Basal release of urokinase plasminogen activator, plasminogen activator inhibitor-1, and soluble plasminogen activator receptor from separated and cultured endometriotic and endometrial stromal and epithelial cells

Christine Bruse, M.D., Ph.D.,[a] *Yongmei Guan, M.D.,*[a,b] *Magdalena Carlberg, Ph.D.,*[a,c] *Kjell Carlström, Ph.D.,*[a] *and Agneta Bergqvist, M.D., Ph.D.*[a,d]

[a] Department of Clinical Science, Unit of Obstetrics and Gynecology, Karolinska University Hospital, Stockholm, Sweden; [b] Department of Obstetrics and Gynecology, The Second Hospital, Harbin Medical University, Beijing, China; [c] Stockholm Center of Public Health, Unit for Psychological Health, Stockholm, Sweden; and [d] Department of Obstetrics and Gynecology, Karolinska Institutet, Danderyds Hospital, Stockholm, Sweden

Objective: To investigate whether separated and cultured endometriotic and endometrial stromal and epithelial cells release urokinase plasminogen activator (uPA), plasminogen activator inhibitor-1 (PAI-1), and soluble plasminogen activator receptor (suPAR) antigens in vitro.
Design: In vitro study.
Setting: University hospital clinic.
Patient(s): Regularly menstruating women with and without endometriosis.
Intervention(s): Tissue samples were collected at surgery performed for clinical reasons.
Main Outcome Measure(s): The antigen concentrations of uPA, PAI-1, and suPAR in culture medium were assayed by enzyme-linked immunosorbent assay.
Result(s): 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. Furthermore, the release of PAI-1 was significantly higher from endometriotic cells than from endometrial stromal cells.
Conclusion(s): This study has demonstrated the basic capacity of separated epithelial and stromal cells from all three types of tissue to release uPA, PAI-1, and suPAR without any paracrine influence, as in vivo. The higher release of PAI-1 from endometriotic stromal cells might have importance for the invasive growth. (Fertil Steril® 2005;83(Suppl 1):1155–60. ©2005 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, endometrium, epithelial cells, stromal cells, PAI-1, suPAR, uPA

Endometriosis, defined as ectopic endometrium, has invasive properties. Regurgitated menstrual debris reaches the pelvic cavity and can locally implant any single structure in the pelvis (1). Endometriosis can also invade distantly. Endometriotic lesions are found outside the pelvic cavity, that is, in the intestines, diaphragm, kidney, somatic muscles, lungs, and pleural cavity, as well as in surgical wounds (2). Although not malignant, the tissue can invade and damage other tissues.

Early endometriosis invades the extracellular matrix (3), and collagen gel invasion in vitro studies have shown that the invasive potential of endometriotic cells is comparable with that of cell lines from a metastatic carcinoma (4).

Received April 14, 2004; revised and accepted September 30, 2004.
Supported by grants from Anders Otto Swärd's Stiftelse, Sigurd och Elsa Goljes Minne, Ragnhild och Einar Lundströms Minne, and the Swedish Medical Research Council (grant no. 17X-09511), Stockholm, Sweden.
Reprint requests: Christine Bruse, M.D., Ph.D., Department of Obstetrics and Gynecology, Karolinska University Hospital, Huddinge, SE-141 86 Huddinge, Sweden (FAX: 46-8-585-875-75; E-mail: christine.bruse@hs.se).

The plasminogen-activating system, including the plasminogen activators (PAs) and their inhibitors (PAIs) and the urokinase plasminogen activator receptor (uPAR), is involved in tissue degradation and remodeling under both normal and pathological conditions (5). Besides being involved in tumor growth, invasion, and metastasis, the plasminogen-activating system also appears to be involved in other cancer cell–directed tissue-remodeling processes, such as angiogenesis, stimulation of fibroblast proliferation, and extracellular matrix (ECM) protein synthesis (6). The activation of plasminogen, leading to the formation of plasmin, is catalyzed by uPA when bound to uPAR or by tissue-type plasminogen activator (tPA). Urokinase PA binds to uPAR with high affinity, and receptor binding of uPA initiates pericellular proteolysis and cell migration, two processes that prepare for tissue invasion (5, 6).

There are two types of uPA receptors, cell surface uPAR and soluble uPAR (suPAR), which arise by alternative splicing of the glycosyl phosphatidyl inositol anchor (7). Soluble uPAR has retained uPA-binding capacity and is a water-

soluble, secreted protein. The function of suPAR is still unknown, but some reports suggest that suPAR can increase the local availability and activity of uPA by retarding its inhibition by PAI-1 and its clearance (8).

The highly potent protease plasmin is able to degrade a broad spectrum of matrix and basement membrane proteins, like fibronectin, laminin, and proteoglycan (9). Plasmin also activates zymogens of other matrix-degrading proteases, like collagenases, stromolysins, and elastases, that is, matrix metalloproteinases (MMPs) (10). Further, it catalyzes activation of latent transforming growth factor-β (TGF-β), which is important for the up-regulation of PAI-1 (9, 11).

We have previously shown significantly elevated levels of uPA and PAI-1 antigens in homogenates from endometriotic and endometrial tissue from women with endometriosis, compared with endometrial tissue from healthy women (12). The levels of uPA and PAI-1 antigen were even significantly higher in endometriotic tissue compared with endometrium from women with endometriosis. We have also recently found that uPA mRNA, PAI-1 mRNA, and uPAR mRNA are up-regulated in different ways in endometriotic tissue and endometrium from women with endometriosis compared with control endometrium (13).

In the present study, we cultured separated and isolated epithelial and stromal cells, respectively, from endometriotic and endometrial tissue from women with and without endometriosis to find out what endogenous potential each of these cell types has to release uPA, PAI-1, and suPAR antigens without any influence of exogenous hormones or growth factors (GF) added, with the exceptions of manufactory, supplemented GFs in the culture medium or by other paracrine interactions.

MATERIALS AND METHODS

Tissue Sources

Endometriotic samples were obtained at laparoscopy for clinical reasons from nine women (mean age, 39 years; range, 26–49 years) with stage III ovarian endometriomas according to American Fertility Society (AFS) revised classification (14). From six of these women (mean age, 40 years; range, 28–49 years), an endometrial sample was obtained simultaneously.

Altogether, 13 endometrial samples from women with endometriosis, AFS stage III (mean age, 37 years; range, 26–49 years) were obtained by uterine curettage performed for clinical reasons. Eight of the women were in the proliferative phase, and five in the secretory phase. Thirteen endometrial samples were used for epithelial cell culture, and 11 samples were used for stromal cell culture.

Control endometrial samples were obtained by uterine curettage from 14 healthy women (mean age, 39 years; range, 30–52 years) undergoing laparoscopic sterilization. Eight of the women were in the proliferative phase, and six in the secretory phase. Of these, 14 samples were used for stromal cell culture and 13 samples were used for epithelial cell culture.

All women were regularly menstruating, and none had taken any sex steroid hormones or had been pregnant or breast-feeding the previous 3 months before surgery. The local ethics committee at Huddinge University Hospital approved the study, and the women gave their oral informed consent for the samples to be collected. Institutional Review Board approval is not required in Sweden.

Sample Preparation and Cell Isolation

Tissue sampling and separation in epithelial and stromal cells was performed as previously described by Guan et al. (15). Briefly, the endometriotic tissue was scraped with the back edge of a knife from the inside of the cysts, which were 2–6 cm in diameter. The endometriotic and endometrial tissue samples were collected immediately after surgical extirpation and transported in cold phosphate-buffered saline (PBS) to the laboratory. Part of the fresh tissue was fixed in 4% formalin, paraffin embedded, sectioned, and stained with hematoxylin-eosin for light microscopic verification of the diagnosis and of the cycle phase, according to Noyes et al. (16).

The fresh tissue was cut into small pieces of 1 mm^3 and transferred into 0.25% type IA collagenase in Dulbecco's Modified Eagle Medium nutrient mixture F-12 (DMEM/F-12) without phenol red, containing 10% fetal bovine serum (FBS), 1% of 1,000 IU/mL penicillin, 1,000 μg/mL streptomycin, and 1% glutamine. Digestion was performed for 1 hour at 37°C under gentle shaking. After digestion, the suspension was filtered through a 100-μm cell strainer to remove mucous material, debris, and undigested tissue. The cells were freed from collagenase by centrifugation (5 minutes, $\times$400 *g*). The pellet was resuspended in DMEM/F-12 and centrifuged at $\times$55 *g* for 2 minutes to pellet epithelial cells.

The epithelial cell pellet was washed in DMEM/F-12 and centrifuged at $\times$55 *g* for 2 minutes, and the new pellet was resuspended in 5 mL DMEM/F-12. Filtration of the epithelial cell suspension was then carried out through a 40-μm sieve (Becton Dickinson Lab Ware, Franklin Lakes, NJ), 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 DMEM/F-12 and again centrifuged at $\times$55 *g* for 2 minutes.

Culture of Endometriotic and Endometrial Stromal Cells

The supernatant, which contained isolated stromal cells, was transferred into a 25-cm^2 plastic culture flask. After one passage to get rid of macrophages, the cells were seeded at 50,000 cells/well (24-well plates) and an adhesion period of 1 week followed when the cells were incubated with DMEM/F-12 containing 10% FBS. The cells were incubated