Evidence for an increased release of proteolytic activity by the eutopic endometrial tissue in women with endometriosis and for involvement of matrix metalloproteinase-9

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BACKGROUND: For the implantation of endometrium in ectopic locations, remodelling of the extracellular matrix (ECM) is necessary. Many studies have shown an increased expression of various proteases in the ectopic endometrium of women with endometriosis. Few, however, have addressed possible changes in protease expression in the eutopic endometrium. METHODS AND RESULTS: Herein, we reveal an increased release of proteolytic activity by the eutopic endometrium of women with endometriosis compared with normal women (P < 0.01). Using zymography and western blotting, we identified matrix metalloproteinase (MMP)-2 and MMP-9 in the culture medium, and further found that MMP-9 secretion, as assessed by zymography and enzyme-linked immunosorbent assay (ELISA), was elevated in women with endometriosis compared with normal women (P < 0.05). No statistically significant difference in MMP-2 secretion between women with and without endometriosis was noted. However, a significant difference in the levels of the tissue inhibitor of metalloproteinases (TIMP)-1, a known MMP-9 inhibitor, was found (P < 0.05). CONCLUSION: The endometriosis-associated increase in proteolysis and imbalance between the secretion of MMP-9 and that of its natural inhibitor, TIMP-1, revealed in the culture medium of endometrial tissue may reflect in vivo the enhanced capacity of this tissue to break down the ECM in host tissues, thereby favouring its ectopic implantation and development.

Key words: endometriosis/MMP-9/proteolysis/TIMP-1

Introduction

Endometriosis is a common gynaecological disorder affecting 10% of the female population during their reproductive life (Strathy et al., 1982). The most widely accepted theory to explain peritoneal endometriosis is Sampson’s theory of retrograde menstruation (Sampson, 1927). According to this theory, the disease arises from ectopic implantation and growth of endometrial tissue that reaches the peritoneal cavity by tubal reflux. In order for ectopic implantation and growth to occur, endometrial tissue must first attach itself to the host tissue, invade it and then obtain from the local vasculature its own blood supply (Giudice et al., 1998). Degradation of the extracellular matrix (ECM) is therefore a basic step in the formation of new vessels in angiogenesis (Yasunaga et al., 1989; Koblizek et al., 1998) with regard to tissue remodelling. Many factors are important for the degradation of ECM and the implantation of endometrial tissue in ectopic sites, notably cathepsin D (Suzumori et al., 2001) and plasminogen (Sillen et al., 2001) and matrix metalloproteinases (MMPs) (Kokorine et al., 1997; Bruner-Tran et al., 2002; Chung et al., 2002).

MMPs form a multigenic family of proteolytic enzymes that depend on zinc for activation (Van Wart and Birkedal-Hansen, 1990; Vallee and Auld, 1992; Rudolph-Owen et al., 1998). They are first secreted in their latent form as proenzymes and can be activated later (Salamonsen et al., 1997). MMPs have different specialities, even if there are considerable overlaps, and together they are able to break down most ECM components, including the different types of collagens that make up the basement membrane (Freitas et al., 1999; Visse and Nagase, 2003). The ECM degradation, operated by the MMPs, is closely regulated by tissue inhibitors of metalloproteinases (TIMPs) under normal physiological conditions such as tissue repair (Okada et al., 1987), embryogenesis (Pajouh et al., 1991) and menstruation (Osteen et al., 1994). Imbalance between MMP and TIMP expression has been involved in various medical conditions, notably rheumatoid arthritis,
tumour invasion and endometriosis (for a review see Osteen et al., 2003).

Several recent studies highlighted the role of MMPs in endometriosis. Koks et al. (2000) reported that MMP activity in menstrual serum is different from and more intense than MMP activity in peritoneal fluid, and that these enzymes may be involved in the early invasion of menstrual endometrium into the ECM of the peritoneum. Different MMPs, such as MMP-1 (Kokorine et al., 1997), MMP-2 (Wenzl and Heinzl, 1998), MMP-7 (Bruner-Tran et al., 2002) and MMP-9 (Liu et al., 2002), were reported to have increased expression in endometriotic lesions. Interestingly, eutopic endometrium from patients with endometriosis was shown to express higher levels of MMP-2 and membranous type 1 (MT1) MMP, and lower levels of TIMP-2, than the endometrium from normal women (Chung et al., 2002). This highlights possible changes in MMP activity in the eutopic endometrial tissue of endometriosis and suggests an enhanced proteolysis which could play a role in enabling this tissue to implant in ectopic locations. Therefore, the aim of the present study was to investigate whether the endometrial tissue from women with endometrious releases increased proteolytic activity compared with normal subjects, and to identify the MMPs involved in that activity.

Materials and methods

Collection and culture of endometrial tissue

The women recruited in this study provided informed consent for a research protocol approved by the Ethics Board for human research of the Saint-François d’Assise Hospital. Endometriosis was identified during laparoscopy or laparotomy in women consulting for infertility and/or pelvic pain. Patients with endometriosis (n = 18; mean age = 32.2 ± 4.4 years) had no other pelvic condition. Seven women were in the proliferative phase (two at the early–mid and five at the late proliferative phase) and 11 women were in the secretory phase (six at the early–mid and five at the late secretory phase) of their menstrual cycles. The stage of endometriosis was determined according to the revised classification of The American Society for Reproductive Medicine (American Society for Reproductive Medicine, 1997). Eight had endometriosis stage I, eight endometriosis stage II and two endometriosis stage III. Normal women (n = 13; mean age = 36.2 ± 6.4 years) were fertile, requesting tubal ligation, and exhibiting no visible evidence of endometriosis upon laparoscopy. Six patients were in the proliferative phase (three at the early–mid and three at the late proliferative phase) and seven were in the secretory phase (four at the early–mid and three at the late secretory phase) of their menstrual cycles. The cycle phase (proliferative or secretory) was determined based on the patients’ cycle history, serum progesterone and Noyes’s histological criteria (Noyes et al., 1975).

Endometrial biopsies were obtained using a sterile pipette (Unimar Inc., Prodimed, Neuilly-en-Tchelle, France). Samples were placed at 4°C in sterile Hanks’ balanced salt solution (HBSS) (Gibco-BRL, Burlington, Ontario, Canada) containing 100 IU/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin, and then immediately transported to the laboratory. Biopsies used in this study were devoid of any visible blood contamination. Tissues were immediately washed with cold HBSS and cut into 1 mm³ pieces. Six pieces of tissue were put in each well (24-well plates) and incubated for 24 h at 37°C, 5% CO₂ with phenol red-free Dulbecco’s modified Eagle’s medium (DMEM)-F12 (Gibco) containing 100 IU/l penicillin, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin. The culture medium was then collected on ice, centrifuged (4°C, 8 min, 10 000 g) in order to eliminate cell debris, aliquoted, and stored at −80°C for future use. Endometrial tissue explants were recovered, total proteins were extracted as described previously (Bigonnese et al., 2001), and protein concentration was determined using the Bio-Rad DC Protein Assay (Bio-Rad Laboratories Ltd, Mississauga, Ontario, Canada).

Protease assay

Protease activity was determined according to an original procedure described by Millet (1977), which was modified in our laboratory so as to allow the use of small sample volumes and a 96-well microplate reader. Basically, 60 µl samples were incubated with an equal volume of a 0.5% azocasein solution at 37°C for a period of 3 h. An equal volume of cold trichloroacetic acid (10% in phosphate buffered saline (PBS)) was then added, and the samples were centrifuged for 30 min at 8000 g. A 25 µl aliquot of 2 mol/l were then added to 175 µl of supernatant and the optical density was read at 405 nm. Proteolysis was also assessed following incubation of endometrial tissue culture supernatants with a final concentration of sheep polyclonal anti-MMP-9 antibody (1 µg/ml) (Oncogene, San Diego, CA), or recombinant human (rh) TIMP-1 (100 ng/ml) for 1 h at 37°C prior to adding azocasein. Anti-MMP-9 antibody and TIMP-1 concentrations were selected based on the mean amount of MMP-9 found by enzyme-linked immunosorbent assay (ELISA) and dose–response assays (data not shown). The proteolytic activity was extrapolated from a standard curve using trypsin (Gibco-BRL) as reference and expressed in USP (United States Pharmacopeia) units/µg of tissue proteins. Assays were performed twice in duplicate.

Zymography

Protease activity in samples of culture supernatant (40 µl) was analysed by zymography on 7.5% SDS-polyacrylamide gels containing 0.5 mg/ml gelatin (Sigma-Aldrich Canada Ltd, Oakville, Ontario, Canada) under non-reducing conditions. Culture supernatant from the human fibrosarcoma HT1080 cell line known for releasing elevated proteolytic activity was used as positive control (a gift from Dr Éric Petitclerc, Québec City, Canada). After electrophoresis, gels were washed with post-electrophoretic buffer (50 mmol/l Tris, 5 mmol/l CaCl₂, 0.02% Na₃) containing 2.5% Triton X-100 (2 × 20 min), then incubated with a post-electrophoretic buffer containing 1% Triton X-100 (20 min at room temperature) and finally incubated overnight at 37°C. Gels were stained with 0.25% Coomassie brilliant blue G-250 dye for 30 min and destained in 30% methanol/10% glacial acetic acid. Quantification of detectable gelatinases was achieved by computer-assisted densitometry (BioImage, Visage 110s, Genomic Solutions Inc., Ann Arbor, MI). Data were normalized to equal endometrial tissue explant proteins (10 µg) which is the mean amount of proteins corresponding to 40 µl of culture supernatant, and expressed as a percentage of control (HT1080 gelatinases). Assays were performed twice in duplicate.

Western blotting

A western blot analysis was used to confirm the presence of MMP-2, MMP-9 and TIMP-1 in explant supernatant. Briefly, proteins in a 40 µl sample of culture were denatured and separated by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) in 10% acrylamide slab gels then transferred onto 0.45 µm nitrocellulose membranes (Protran, Schleicher & Schuell, Keene, NH). Nitrocellulose membranes were cut into strips and incubated overnight at 4°C with a monoclonal mouse anti-human MMP-2 antibody (Oncogene Research
Products, Boston, MA) at 1 µg/ml of blocking solution, a monoclonal mouse anti-human MMP-9 antibody (Oncogene Research Products) at 2 µg/ml of blocking solution, a monoclonal mouse anti-human TIMP-1 antibody (R & D Systems, Minneapolis, MN) at 2 µg/ml of blocking solution [0.1 mol/l Tris buffer, 0.9% NaCl/0.05% Tween-20 containing 5% non-fat dry milk (w/v)] or with normal mouse immunoglobulins (IgGs) at the same concentrations. The strips were then washed in Tris-buffered saline (TBS)–0.1% Tween-20, incubated for 1 h at 37°C with a biotin-conjugated goat anti-mouse antibody (Jackson ImmunoResearch Laboratories, West Grove, PA), diluted 1:10 000 in the blocking solution, washed again in TBS–0.1% Tween-20, incubated for 30 min at 37°C with peroxidase-conjugated streptavidin (Jackson ImmunoResearch Laboratories) and diluted 1:5000 in the blocking solution. After a final wash in TBS–0.1% Tween-20, strips were incubated for 1 min with an enhanced chemiluminescence system using BM chemiluminescence blotting substrate (POD) (Roche Diagnostics, Laval, Quebec, Canada), and exposed to Kodak BioMax film for several time intervals, thus allowing for optimal detection (all bands visible but not overexposed).

Measurement of MMP-2, MMP-9 and TIMP-1

MMP-2, MMP-9 and TIMP-1 concentrations in the explant culture medium were measured using an ELISA procedure developed in the laboratory. For the assay measuring MMP-2, we used a mouse monoclonal anti-human MMP-2 antibody (Chemicon International Inc., Temecula, CA) and a rabbit polyclonal anti-human MMP-2 antibody (Oncogene Research Products). For the assay measuring MMP-9, we used a mouse monoclonal anti-human MMP-9 antibody and a sheep polyclonal anti-human MMP-9 antibody (Oncogene Research Products). Finally, for the TIMP-1 assay, we used a mouse monoclonal anti-human TIMP-1 antibody (R & D Systems). Basically, 96-well plates were coated overnight at 4°C with the respective mouse monoclonal antibody. Plates were washed four times with a PBS buffer containing 0.1% Tween-20 (washing buffer). Aliquots (70 µl) of rhMMP-2, rhMMP-9 (Oncogene Research Products) and rhTIMP-1 (R & D Systems), at concentrations ranging from 100 pg/ml to 7.68 ng/ml, diluted in DMEM-F12 culture medium supplemented with 0.5% bovine serum albumin (BSA), or non-diluted samples were then added to the plates. After a 60 min incubation period set at 37°C, the plates were washed and incubated for 60 min at 37°C with their corresponding antibody; either the MMP-2, MMP-9 or TIMP-1 polyclonal antibodies diluted in PBS containing 0.5% BSA. After a further wash, the plates were incubated at 37°C for a further 60 min with a biotin–streptavidin-conjugated goat anti-rabbit AffiniPure IgG for MMP-2, a biotin–streptavidin-conjugated donkey anti-sheep AffiniPure IgG for MMP-9, and a biotin–streptavidin-conjugated rabbit anti-goat AffiniPure IgG for TIMP-1 (Jackson ImmunoResearch Laboratories). Another washing was done before the plates were incubated at 37°C for 45 min with peroxidase-conjugated streptavidin (Jackson ImmunoResearch Laboratories). After a final wash, 70 µl of TMB (3,3',5,5'-tetramethylbenzidine)-peroxidase substrate (Bio-Rad Laboratories Ltd, Mississauga, Ontario, Canada) was added to each well. The enzymatic reaction was terminated by adding 50 µl of 1 mol/l H2SO4, the optical density was determined at 450 nm. MMP-2, MMP-9 and TIMP-1 concentrations were calculated by interpolating the corresponding standard curves. Assays were performed twice in duplicate. Data were normalized and expressed as ng/g of total tissue proteins. The sensitivity limit of these assays was 1 ng/ml for MMP-2, 0.6 ng/ml for MMP-9, and 0.4 ng/ml for TIMP-1, with intra- and inter-assay coefficients of variation of 2.9 and 3.1% for MMP-2, 2.3 and 3.5% for MMP-9, and 3.0% and 3.1 for TIMP-1, respectively.

Statistical analysis

Data followed a normal distribution, and were therefore expressed as mean ± SEM. An unpaired t-test was used for comparisons of means using GraphPad Software, Prism 3.0 (GraphPad Software, San Diego, CA). Differences were considered as statistically significant whenever a P-value <0.05 occurred. The statistical power for each analysis was performed using GraphPad StatMate software.

Results

Endometrial tissue from women with endometriosis releases increased proteolytic activity

Fresh explants of endometrial tissue from women with endometriosis and from healthy controls were cultured in a fetal bovine serum (FBS)-free medium for 24 h and the proteolytic activity released in the culture medium was assessed using a modified quantitative azocasein-based protease assay. As shown in Figure 1A, endometrial tissue from patients with endometriosis released significantly more proteolytic activity than tissue from normal controls (P < 0.01). Furthermore, this was noticeable and statistically significant in both the proliferative and the secretory phases of the menstrual cycle (P < 0.05) (Figure 1B).

Identification of MMP-2 and MMP-9 and quantification of their levels in endometrial tissue culture supernatants

Culture supernatants of endometrial tissue were first analysed by gelatinolytic zymography. As shown in Figure 2A, five distinct bands of gelatinase activity at 170, 92, 86, 72 and 66 kDa were found. The 92 and 86 kDa bands are consistent with the MMP-9 latent and active forms, respectively, whereas the 72 and 66 kDa bands are consistent with the MMP-2 latent and active forms. The 170 kDa band might correspond to the putative dimeric MMP-9 pro-form described previously (Watari et al., 1999). Western blot analysis of the culture supernatant of endometrial tissue using anti-MMP-9 antibody confirmed the presence of the 86 kDa active and the 92 and 170 kDa latent forms of MMP-9. No immunoreactive bands were observed in any of the blots when the primary antibody was replaced with an equal concentration of mouse immunoglobulin of the same isotype (data not shown). The anti-MMMP-2 antibody detected two bands whose molecular weights (66 and 72 kDa) were consistent with the active and latent forms of MMP-2 described earlier. Two other bands of higher molecular weight (90 and 200 kDa) were also detected. These bands may correspond to other forms of pro-MMP-2 not detectable by zymography (Figure 2B).

Densitometry analysis of MMPs’ lysis bands showed that both pro- and putative dimeric MMP-9 bands were significantly more intense in women with endometriosis than in normal women (P < 0.05) (Figure 3A). A tendency for an increased intensity for both MMP-9 bands was observed in the proliferative (P = 0.13 and P = 0.13, respectively) and the secretory (P = 0.10 and P = 0.09, respectively) phases of the menstrual cycle. However, these differences were not statistically significant. The intensity of the 86 kDa active MMP-9 band was generally weak and was often not measurable by densitometry. No statistically significant difference in the
intensity of MMP-2 lysis bands between women with and without endometriosis was observed, but a slight tendency for increased active MMP-2 levels in endometriosis could be seen ($P = 0.18$) (Figure 3B); however, this was not significant.

MMP-9 and -2 secretion by endometrial tissue was assessed further by ELISA. As shown in the Figure 4A, MMP-9 secretion was higher in women with endometriosis than in normal controls ($P < 0.01$). Furthermore, this was observed in both the proliferative and the secretory phases of the menstrual cycle ($P < 0.05$). However, no statistically significant difference in MMP-2 secretion between women with and without endometriosis was noted ($P = 0.48$) (Figure 4B).

Identification and quantification of TIMP-1 levels

The finding of an endometriosis-associated increase in MMP-9 secretion in the culture supernatant of endometrial tissue prompted us to assess the presence of TIMP-1, a known inhibitor of MMP-9, and to quantify its secretion in women with and without endometriosis. Western blot analysis showed a major 31 kDa band, which corresponds to the known molecular weight of the inhibitor, and two minor bands of 40 and 6.5 kDa. The nature of the 40 kDa band remains unknown as yet, but the 6.6 kDa band, which is present in the rhTIMP-1 used as reference, could possibly represent some form of TIMP-1 degradation (Figure 5A).

Measurement of TIMP-1 concentrations in the culture supernatants by ELISA and statistical analysis of the data revealed a significant diminution of TIMP-1 secretion in women with endometriosis as compared with normal women ($P < 0.05$) (Figure 5B). Interestingly, such a decrease in TIMP-1 secretion was found to occur in the secretory phase of the menstrual cycle ($P < 0.01$), whereas no statistically significant difference in the proliferative phase was noted ($P = 0.59$).

Inhibition of proteolysis

To support the evidence that increased proteolytic activity of endometrial tissue from women with endometriosis involves
MMP-9, we further assessed the effect of MMP-9 inhibition on proteolysis. As shown in Figure 6, incubation of endometrial tissue culture supernatant with rhTIMP-1 resulted in a significant inhibition of azocasein proteolysis in normal women and women with endometriosis ($P < 0.05$ and $P < 0.01$, respectively). Incubation of endometrial tissue culture supernatants with anti-MMP-9 antibody did not result in a statistically
significant inhibition of azocasein proteolysis in normal women or women with endometriosis, but a tendency for a reduced proteolysis could be seen ($P = 0.11$ and $P = 0.09$, respectively). No effect on azocasein proteolysis was observed when the anti-MMP-9 antibody was replaced with an equivalent concentration of normal sheep IgGs (data not shown).

Discussion

The most widely accepted theory for the explanation of endometriosis is the transplantation theory by which auto- logous cells can implant and develop in ectopic locations. For implantation of these cells to be successful, a breakdown of the ECM must occur. Several recent studies underlined the role of proteases in the capability of endometrial tissue to invade and develop into host tissues (Sharpe-Timms and Cox, 2002). However, only a few addressed possible changes in protease expression in the eutopic endometrium in endometriosis patients (Chung et al., 2001, 2002).

Our present study showed an increased release of proteolytic activity by the eutopic endometrial tissue of women with endometriosis compared with normal women. Furthermore, statistical analysis of our data with regard to the influence of the menstrual cycle showed such an increase to occur in both the proliferative and the secretory phases. These data are in keeping with our previous findings showing a significant increase in cytokine secretion in proliferative as well as secretory phase endometrial tissue in women with endometriosis (Jolicoeur et al., 1998), and point toward a process of local cell activation occurring throughout the menstrual cycle of patients.

The source and nature of factors involved in such an increased protease release by the endometrial tissue of endometriosis patients remain unclear. It is well documented that endometriosis is associated with immuno-inflammatory changes in the eutopic endometrium (Lebovic et al., 2001; Sharpe-Timms, 2001) with, for instance, an increased auto-immune response (Lebovic et al., 2001), leukocyte infiltration (Witz et al., 1994; Ota et al., 1996) and secretion of proinflammatory mediators, such as complement component 3, monocyte chemotactic protein-1 (MCP-1), interleukin (IL)-1 and IL-6 (Sharpe-Timms, 2001). Consequently, proteases can be released by immune cells, as well as by endometrial cells, at least in response to local inflammatory stimuli.

In the present study, further analysis of endometrial tissue culture media by zymography allowed detection of MMP-2 and MMP-9, and revealed an increased release of MMP-9 in endometriosis as assessed by densitometric analysis of the lysis bands corresponding to the pro- and putative dimeric MMP-9. Measurement of MMP-9 concentrations by ELISA corroborated the zymography data and showed a significant increase in MMP-9 secretion by the endometrial tissue of women with endometriosis. Interestingly, such a secretion followed a pattern comparable with that of the proteolytic activity regarding the influence of the menstrual cycle and was found to be elevated in the proliferative and the secretory phases. Moreover, adding TIMP-1, a natural inhibitor of several MMPs including MMP-9 (Goldberg et al., 1992; Hanemaaijer et al., 1993), and a neutralizing anti-MMP-9 antibody to endometrial tissue culture media led to a noticeable inhibition of proteolysis. This substantiates the involvement of MMP-9 in the increased proteolytic activity found in women with endometriosis, and makes its role in endometriosis development plausible.

Several recent studies stressed the role of MMP-9 in tumour invasion and metastasis (Itoh et al., 1999; Kondraganti et al., 2000; Turner et al., 2000). In the human endometrium, MMP-9 has been located in leukocytes, glandular epithelial cells and developing arterioles (Jezierska et al., 1996; Freitas et al., 1999), and was reported to be involved in vascular growth and angiogenesis. In endometriosis, a higher gelatinase activity was found in the endometriotic tissue as compared with eutopic endometrial tissue (Liu et al., 2002). Studying MMP-9 at the mRNA level, Chung et al. (2001) demonstrated that there was increased expression in the ectopic versus the eutopic endometrial tissue. However, no significant differences were reported between women with and without endometriosis with regard to MMP-9 mRNA expression in the eutopic endome trium. Using zymography and a quantitative sensitive ELISA procedure, our present study showed an increased release of MMP-9 protein by the endometrial tissue in women with endometriosis. Studies regarding MMP-9 protein expression in situ in the endometrial tissue of women with and without endometriosis are currently in progress in our laboratory.

Natural tissue inhibitors of MMPs, TIMPs, are especially important in regulating ECM remodelling since they regulate MMP activity. Reduced TIMP expression appeared to be involved in cancer invasiveness (Khokha et al., 1989; Ponton et al., 1991). TIMP-1 is responsible for regulating the active forms of MMP-1, MMP-3 and MMP-9, because the formation of a complex between the inhibitor with any of these MMPs leads to protease inactivation (Hanemaaijer et al., 1993). In addition, TIMP-1 has the capacity to form a complex with pro- MMP-9, thereby blocking the activation of the enzyme (Goldberg et al., 1992; Hanemaaijer et al., 1993). Interestingly, TIMP-1 concentration was found to be decreased in the peritoneal fluid of endometriosis patients compared with normal controls (Sharpe-Timms et al., 1998). Interestingly, our present data are consistent with these findings and reveal a significant decrease in TIMP-1 secretion by the endometrial tissue of women with endometriosis as compared with normal women. Furthermore, such a decrease appeared to be more marked and statistically significant in the secretory phase of the menstrual cycle. This denotes an imbalance between MMP-9 and TIMP-1 levels, particularly in the secretory phase, which may play an important role in the invasiveness of endometrial tissue from women with endometriosis and its ability to implant in ectopic locations. Chung et al. (2001) also found an imbalance between MMP-9 mRNA levels in the eutopic endometrium of endometriosis patients and those of another of its natural inhibitors, TIMP-3, with an increased MMP-9/ TIMP-3 ratio. This, together with our findings, suggests a deregulation of MMP-9 secretion by the endometrial tissue in endometriosis patients, an inadequate control of its activity and a possible role for this proteolytic enzyme in the aptitude of endometrial cells for remodelling and implanting into host tissues.
Using a quantitative ELISA procedure and densitometric analysis by zymography, our present study failed to show any significant difference in MMP-2 secretion between endometrial tissues in women with and without endometriosis. The role of MMP-2 in endometriosis is still a matter of debate. According to Wenzl and Heinzl (1998), the ectopic endometrium has a significantly higher capacity to produce the latent forms (72 kDa) of MMP-2 as compared with the uterine endometrium. However, in another study, ectopic endometrium appeared to express significantly less MMP-2 mRNA than did eutopic endometrium in women with endometriosis during both the proliferative and the secretory phases of the menstrual cycle (Chung et al., 2002). Available data pertaining to MMP-2 expression in the eutopic endometrial tissue in women with and without endometriosis also remain contradictory. By means of an immunohistochemical evaluation of MMP-2 expression in endometrial tissue, Wenzl and Heinzl (1998) showed no significant difference between women with and without endometriosis; only ectopic endometrial tissue was found to have an enhanced expression of MMP-2. However, another recent study showed an increased expression of MMP-2 in the eutopic endometrium, at both the protein and mRNA levels, as assessed by zymography and quantitative RT–PCR, respectively (Chung et al., 2002).

In conclusion, the present study revealed an increased release of proteolytic activity by the eutopic endometrium in women with endometriosis as compared with normal women regardless of the menstrual cycle phase, identified MMP-2 and MMP-9 in the culture medium, and further showed concurrent elevation of MMP-9 secretion in women with endometriosis throughout the menstrual cycle. On the other hand, the endometrial tissue secretion of TIMP-1, a natural inhibitor of MMP-9, was significantly reduced in women with endometriosis as compared with normal women, particularly in the secretory phase of the menstrual cycle. The endometriosis-associated increase of proteolysis and imbalance between MMP-9 secretion and that of its natural inhibitor TIMP-1 revealed in the culture medium of endometrial tissue may reflect in vivo the enhanced capability of this tissue to invade and break down the ECM in host tissues, thereby favouring its ectopic implantation and development. Work is in progress in our laboratory in order to determine the nature of other possibly overproduced proteases.

References


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