Increased soluble interleukin-1 receptor type II proteolysis in the endometrium of women with endometriosis

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Numerous functional changes were observed in the intrauterine endometrial tissue of women with endometriosis. Our previous studies revealed a marked decrease in the expression of interleukin-1 receptor type 2 (IL-1RII), a decoy receptor known for its ability to buffer IL-1 effects. The aim of the present study was to assess whether post-translational mechanisms such as proteolysis may contribute to the down-regulation of IL-1RII levels. Our data showed that soluble IL-1RII (sIL-1RII) concentrations released by freshly cultured endometrial tissue were significantly lower in women with endometriosis than in normal women (P < 0.01) and further revealed a statistically significant correlation between increased proteolysis and decreased sIL-1RII levels (P < 0.05; r = −0.47). 125I-labelled soluble recombinant human IL-1RII ([125I]srhIL-1RII) was significantly more degraded in culture supernatant of tissues from women with endometriosis compared to normal women (P < 0.05), and natural tissue inhibitor of matrix metalloproteinase (TIMP)-1 inhibited 125I[srhIL-1RII] degradation. Incubation of srhIL-1RII with active rhMMP-9 resulted in a dose-dependent degradation of srhIL-1RII as analysed by western blotting. Dual immunofluorescence showed an increased immunostaining for matrix metalloproteinase-9 in situ in the endometrial tissue of women with endometriosis compared to normal women and a decreased immunostaining for IL-1RII. The present study showed a reduced release of sIL-1RII by the endometrial tissue of women with endometriosis and revealed a proteolytic post-translational mechanism which may be involved in the down-regulation of IL-1RII levels. This may enhance IL-1-mediated activation of endometrial cells and contribute to the local immuno-inflammatory process observed in endometriosis patients.

Key words: endometriosis/endometrium/IL-1RII/proteolysis/MMP-9

Introduction

Endometriosis is one of the most common gynaecological diseases in women of reproductive age and frequently associated with infertility and pelvic pain (Strathy et al., 1982; Goldman and Cramer, 1990). The disease is characterized by an abnormal development of endometrial tissue outside the uterus, mainly in the peritoneal cavity, and associated with an aberrant immuno-inflammatory process that takes place not only in ectopic sites where endometrial tissue abnormally implants, but even in the eutopic intrauterine endometrium (Sharpe-Timms, 2001).

Interleukin-1 (IL-1), a major proinflammatory cytokine, is believed to have an important role in endometriosis pathophysiology. Increased concentrations of IL-1 were found in the peritoneal fluid of endometriosis patients (Fakih et al., 1987). Peripheral blood monocytes as well as peritoneal macrophages of women with endometriosis secrete elevated concentrations of IL-1 (Zeller et al., 1987; Mori et al., 1992). An up-regulation of IL-1 expression was recently observed in the eutopic endometrial tissue of endometriosis patients (Bergqvist et al., 2001). IL-1 induces an angiogenic phenotype in endometriotic cells and stimulates the secretion of vascular endothelial growth factor and IL-6 (Lebovic et al., 2000). According to our own data, both ectopic and eutopic endometrial tissue of women with endometriosis are more responsive to IL-1 and secrete increased amounts of monocyte chemotactic protein-1 (MCP-1), IL-8, and the chemokine regulated upon activation, normal T cell expressed and secreted (RANTES) (Akoum et al., 1995a,b, 2001b, 2002). Interestingly, our subsequent studies revealed a marked decrease in the expression of IL-1 receptor type II (IL-1RII) in the eutopic endometrial cells of endometriosis women (Akoum et al., 2001a), whereas no significant change in IL-1RII expression was noted (unpublished data). Evidence available to date indicates that IL-1 exerts its biological effects via IL-1RI, the functional signalling receptor, whereas IL-1RII acts as decoy receptor for IL-1, thereby buffering the cytokine’s effects on target cells (Colotta et al., 1993;
Sims et al., 1993; Greenfeder et al., 1995). Actually, the membrane-bound receptor can be cleaved and released in a soluble form from the cell surface following proteolysis (Orlando et al., 1997; Cui et al., 2003). However, both the soluble and the membrane forms of the receptor keep their ability to bind IL-1 and to neutralize its effects (Dunne and O’Neill, 2003). Consequently, decreased IL-1RII expression in the endometrial tissue of endometriosis patients reveals a deficiency in the capability of endometrial and endometriotic cells to down-regulate IL-1 effects, which may play an important role in the aberrant inflammatory process described in ectopic and eutopic endometrial sites.

In a first attempt to elucidate the mechanisms underlying such a deficiency in IL-1RII protein expression observed in the endometrial tissue of endometriosis patients, we studied IL-1RII mRNA expression and found a significant decrease in women with endometriosis as compared to normal women (Kharfi et al., 2002). This points toward a defect in IL-1RII gene transcription and/or to decreased mRNA stability, and may at least in part explain IL-1RII reduced protein expression. More recently, we found an increased proteolysis and a significant increase in protease release in the culture supernatant of endometrial tissue from women with endometriosis and identified matrix metalloproteinase-9 (MMP-9) as one of the major overproduced proteases (Collette et al., 2004). It is now well documented that proteolysis is one of the major mechanisms involved in the regulation of cytokine activity and inflammation, and the role of MMP in this process has been reported (McQuibban et al., 2000, 2002; Van Den Steen et al., 2000, 2003; Nelissen et al., 2003). It is also known that proteases such as MMP are involved in IL-1RII cleavage and shedding (Orlando et al., 1997; Cui et al., 2003). The objective of the present study was therefore to investigate whether post-translational mechanisms such as proteolysis may be implicated in the degradation of sIL-1RII. This may further reduce sIL-1RII availability, amplify IL-1mediated cell activation and contribute to functional changes in the eutopic endometrium of endometriosis patients.

Materials and methods

Chemicals

Recombinant human (rh) IL-1β, soluble recombinant human IL-1 type II receptor (srhIL-1RII), tissue inhibitor of matrix metalloproteinase (rhTIMP-1) and goat polyclonal anti-srhIL-1RII antibody were purchased from R&D Systems (USA). Peroxidase-conjugated rabbit anti-goat antibody, biotin-conjugated rabbit anti-goat antibody and peroxidase-conjugated streptavidin were supplied by Jackson ImmunoResearch Laboratories, Inc. (USA). Active rhMMP-9 and sheep polyclonal anti-hMMP-9 antibody were obtained from Oncogene research product (USA). Hanks’ balanced salt solution (HBSS) without calcium and magnesium, Dulbecco’s modified Eagle’s medium F-12 (DMEM–F-12) and antibiotics–antimycotics were purchased from Invitrogen Life Technologies (Canada). Monoclonal mouse anti-hMMP-9 antibody was obtained by Novoceastra Laboratories, Inc. (Canada). [125I]Na was supplied by NEN, Perkin Elmer (Canada). Fluorescein isothiocyanate-conjugated streptavidin and rhodamine-conjugated sheep anti-mouse antibody were obtained from Sigma (USA).

IL-1RII enzyme-linked immunosorbent assay (ELISA)

Soluble IL-1RII concentrations were measured according to our previously reported procedure (Kharfi and Akoum, 2001).

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 volume and a 96-well microplate reader as previously described (Collette et al., 2004). 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.

Subjects and tissue collection

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. Cultured endometrial biopsies were from women with endometriosis (n = 17; mean age 32.7 ± 4.6 years) who had no other pelvic condition. Six were at the proliferative phase and 11 at the secretory phase. The stage of endometriosis was determined according to the revised classification of the American Society for Reproductive Medicine (1997). Five had endometriosis stage I, 10 endometriosis stage II and two endometriosis stage III. Control tissues were from normal women (n = 14; mean age = 35.0 ± 5.4 years) who were fertile, receiving tubal ligation, and exhibiting no visible evidence of endometriosis upon laparoscopy. Six patients were at the proliferative phase and eight at the secretory phase. Endometrial biopsies used for immunohistochemical analyses were from 10 women with endometriosis (mean age 32.6 ± 2.6 years) and nine normal women (mean age 34.6 ± 5.5 years). Five women with endometriosis were at the proliferative phase and five at the secretory phase. Six had endometriosis stage I and four endometriosis stage II. Five biopsies from normal women used for immunohistochemistry were at the proliferative phase and four at the secretory phase. The cycle phase (proliferative or secretory) was determined based on the patient’s cycle history, serum progesterone, and histological criteria of Noyes et al. (1975). Endometrial samples were collected with a curette before laparoscopy. The tissue was placed in cold, sterile HBSS containing 1% antibiotics, then immediately transported to the laboratory. A part of the biopsy was taken for explant culture, and the remaining was snap-frozen with Tissue-Tek OCT compound (Miles, Inc., USA) and stored at −80°C until analysed by immunohistochemistry.

Culture of endometrial tissue

Biopsies used in this study were devoid of any visible blood contamination. Tissues were immediately washed with cold HBSS and cut into pieces of ~1 mm³. 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 DMEM–F–12 medium containing 100 IU/ml penicillin, 100 mg/ml streptomycin, and 0.25 mg/ml amphotericin. The culture medium was then collected on ice, centrifuged in order to eliminate cell debris, aliquoted, and stored at −80°C for future use. Endometrial tissue explants were recuperated, and 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, Canada).
MMP-9 ELISA
MMP-9 concentrations in the explant culture medium were measured using an ELISA procedure developed in the laboratory as previously described (Collette et al., 2004).

Evaluation of radiolabelled srhIL-1RII degradation
Radiolabelling of carrier-free srhIL-1RII with [125I]Na was conducted using the chloramine-T method (Hunter and Greenwood, 1962) and stored at 4°C in the presence of 0.02% NaNO3 and 0.1% bovine serum albumin (BSA). Radiolabelled srhIL-1RII (12.500 cpm) in a total volume of 5 μl phosphate-buffered saline (PBS) – 0.1% BSA were incubated in the presence of 25 μl of culture supernatants at 37°C for 1–6 h. Radiolabelled srhIL-1RII incubated or not with the culture medium for the same time-periods were included as controls. The reaction was stopped by heating samples in 5X loading buffer [1.25 mol/l Tris–HCl pH 6.8, 50% (v/v) glycerol, 25% β-mercaptoethanol, 10% (w/v) sodium dodecyl sulphate (SDS), and 0.01% (w/v) Bromophenol Blue]. Samples were then separated by SDS–polyacrylamide gel electrophoresis (PAGE) on a 12% (w/v) acrylamide–linear gradient slab gel, fixed and then transferred onto 0.45 μm nitrocellulose membranes. Membrane strips were successively incubated with a polyclonal goat anti-srhIL-1RII antibody (0.1 μg/ml in PBS, 0.1% Tween 20) for 1 h at room temperature, peroxidase-conjugated rabbit anti-goat antibody (1:20000 dilution in PBS, 0.1% Tween-20) for 1 h at room temperature, PBS, Tween 20, an enhanced chemiluminescence reagent (Roche Diagnostic, Canada) for 1 min at room temperature and exposed to BioMax film for 1–2 min for an optimal detection (all bands visible but not overexposed).

Dual immunofluorescent staining
Cryosections (5 μm) of Optimal Cutting Temperature (OCT)-frozen endometrial tissue were mounted on poly-L-lysine-coated microscope glass slides, fixed for 20 min in a 10% buffered formalin phosphate solution (Fisher Scientific, Canada), and washed in PBS. After permeabilization with Triton X-100 (1% in PBS) and elimination of endogenous peroxidase with H2O2 (0.3% in absolute methanol), sections were incubated at room temperature for 90 min with a mouse monoclonal anti-hMMP-9 antibody (1:20 dilution in PBS–BSA 1%). Sections were then incubated at room temperature for 60 min with a goat polyclonal anti-srhIL-1RII antibody (1:7 dilution in PBS–BSA 1%) followed by a 60 min incubation with biotinylated rabbit anti-goat antibody (1:100 in PBS–BSA 1%). After a subsequent wash in PBS, tissue sections were incubated simultaneously for 60 min at room temperature in the dark with fluorescein isothiocyanate-conjugated streptavidin (diluted 1:100 in PBS–BSA 1%) and rhodamine-conjugated sheep antiumouse antibody (diluted 1:10 in PBS–BSA 1%). After a final wash in PBS, slides were mounted in Mowiol containing 10% p-phenylenediamine (Sigma, USA), an antifading agent, and observed under the microscope (Olympus) equipped for fluorescence with a 100 W UV lamp. Images were taken using a M300 digital camera (JAI) and analysed with Isis 4.4.25 software.

Statistical analysis
Data followed a parametric distribution, and were therefore expressed as mean ± SEM. An unpaired t-test was used for comparing those means and analysis of correlation was carried out using the Pearson correlation coefficient. All analyses were performed using GraphPad Software, Prism 3.0 (GraphPad Software, USA). Differences were considered as statistically significant for P < 0.05.

Results
Soluble IL-1RII release by the endometrial tissue of normal and endometriosis women
Fresh explants of endometrial tissue from normal and endometriosis women were cultured for 24 h and the amount of sIL-1RII released in the culture medium was determined by ELISA. Data shown in Figure 1A show that sIL-1RII concentrations were significantly lower in women with endometriosis than in normal women (P < 0.01). Western blot analysis of sIL-1RII in endometrial tissue culture supernatant showed a major 45 kDa band whose mol. wt is equivalent to the known mol. wt of the soluble receptor and a series of lower mol. wt bands which may correspond to degraded sIL-1RII. Furthermore, sIL-1RII bands were generally less intense in women with endometriosis than in normal women. A representative western blot is shown in Figure 1B.

Soluble IL-1RII release from the cell surface is known to result from proteolytic cleavage of the membrane-bound receptor (Orlando et al., 1997; Cui et al., 2003). Our data described above suggest that proteases may have the capability of degrading the soluble receptor following its
release, thereby contributing to down-regulating its extracellular levels. Furthermore, our previous data showed that endometrial tissue from women with endometriosis produces and releases higher amounts of proteases than tissue from normal women, and that MMP-9 is one of the main overproduced proteases (Collette et al., 2004). Therefore, in parallel to sIL-1RII concentrations in endometrial tissue culture supernatants, we quantified the proteolytic activity and MMP-9 concentrations and found a significant increase in women with endometriosis compared to normal women ($P < 0.05$ and $P < 0.05$, respectively) (Figure 2), which is in keeping with our previous data (Collette et al., 2004). Interestingly, sIL-1RII concentrations showed a significant inverse correlation with the proteolytic activity ($P < 0.05$; $r = -0.47$) and a trend for an inverse correlation with MMP-9 concentrations ($P = 0.08$; $r = -0.40$). Thus, these data suggest an endometriosis-associated diminution of sIL-1RII stability due to increased proteolysis and possible involvement of MMP-9.

**Soluble IL-1RII stability**

Based on the above data, we further assessed sIL-1RII stability in the culture supernatants of endometrial tissues from women with and without endometriosis. Soluble rhIL-1RII was labelled with $^{[125]}$INa and incubated at $37^\circ$C with culture supernatants for a $3$ h period. This time was required for conspicuous degradation of $^{[125]}$IrhIL-1RII, and was selected on the basis of degradation kinetics carried out with different culture supernatants. Figure 3A shows a representative autoradiogram of $^{[125]}$IrhIL-1RII degradation in endometrial tissue culture supernatants. Data were expressed as the percentage of $^{[125]}$IrhIL-1RII degradation in culture supernatants, taking the basic culture medium as reference ($0\%$ degradation). Statistical analysis of the data showed an increased degradation of $^{[125]}$IrhIL-1RII in the culture supernatants of tissues from women with endometriosis as compared to normal women ($P < 0.05$) (Figure 3B). Statistical analysis of data using the Pearson correlation coefficient showed that $^{[125]}$IrhIL-1RII degradation in women with endometriosis significantly correlated with the increased proteolytic activity observed in these women ($P < 0.05$; $r = 0.57$). Furthermore, a trend for a correlation between $^{[125]}$IrhIL-1RII degradation and increased MMP-9 concentrations was observed ($P = 0.056$; $r = 0.44$).
Involvement of MMP-9 in sIL-1RII degradation

Considering these data, we then assessed whether MMP-9 can effectively degrade sIL-1RII and be involved in the increased degradation of the soluble receptor in women with endometriosis. In fact, available literature indicates the presence of different putative cleavage sites for MMP in sIL-1RII protein sequence. In particular, IL-1RII appears to have two different cleavage sites for MMP-9 situated in positions 139–143 and 237–241 (McMahan et al., 1991; Kridel et al., 2001). To achieve this, we first incubated endometrial tissue culture supernatants with rhTIMP-1, a natural specific inhibitor of several MMP including MMP-9, or with a sheep polyclonal antibody specific to hMMP-9. A representative autoradiogram illustrated in Figure 4A shows that both rhTIMP-1 and anti-hMMP-9 antibody partially blocked [125I]srhIL-1RII degradation in tissue culture supernatants taking the basic culture medium as reference. Values are expressed as a percentage of [125I]srhIL-1RII degradation in tissue culture supernatants as compared to normal controls (P < 0.05).

![Figure 3](https://academic.oup.com/humrep/article-abstract/20/5/1177/2356689)

**Figure 3.** Measurement of srlhIL-1RII degradation in endometrial tissue conditioned medium. (A) Representative autoradiogram of soluble [125I]srhIL-1RII protein degradation. Lanes 1 and 2: [125I]srhIL-1RII incubation with DMEM for 1 and 3 h respectively. Lanes 3 and 4: [125I]srhIL-1RII incubation with tissue conditioned medium from a patient with endometriosis for 1 and 3 h respectively. Lanes 5 and 6: [125I]srhIL-1RII incubation with tissue conditioned medium from a normal patient for 1 and 3 h respectively. (B) Densitometric analysis of [125I]srhIL-1RII bands. Data are expressed as the percentage of [125I]srhIL-1RII degradation in tissue culture supernatants taking the basic culture medium as reference. Values are means ± SEM of duplicate measurements from two different assays. E: endometriosis women (n = 12); N: normal women (n = 10). *Significant difference between endometriosis patients and normal controls (P < 0.05).

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![Figure 4](https://academic.oup.com/humrep/article-abstract/20/5/1177/2356689)

**Figure 4.** Effect of MMP-9 on srhIL-1RII protein degradation. (A) Representative autoradiogram of [125I]srhIL-1RII incubated with tissue culture supernatants from women with endometriosis in the presence of rhTIMP-1 (lane 1), polyclonal anti-hMMP-9 antibody (lane 2) or in the absence of inhibitors (lane 3). [125I]srhIL-1RII incubated with DMEM was taken as control (lane 4). (B) Representative western blot of srlhIL-1RII (10 ng) incubated with different amounts of rhMMP-9 (0, 100, 200, 400 and 600 ng) (lanes 1–5). (C) Representative western blot of srlhIL-1RII (5 ng) incubated with the vehicle buffer alone (lane 1), rhMMP-9 (200 ng) and rhTIMP-1 (40 ng) (lane 2), rhMMP-9 (200 ng) and polyclonal anti-hMMP-9 antibody (4 μg) (lane 3) or rhMMP-9 alone (200 ng) (lane 4).

**Discussion**

The present study showed that human endometrial tissue releases sIL-1RII. Western blot analysis showed a major 45 kDa band and a number of lower mol. wt bands which may correspond to degraded sIL-1RII. The study further showed that sIL-1RII concentrations released by the endometrial tissue were markedly lower in women with endometriosis than in normal women, which is in keeping with our previous data showing a marked decrease in IL-1RII immunostaining in situ in the endometrial tissue of women with endometriosis (Akoum et al., 2001a) and a significant diminution in IL-1RII mRNA levels (Kharfi et al., 2002).

Soluble IL-1RII is found in the general circulation under physiological conditions (Juffermans et al., 1998; Laurincova, 2000). Elevated levels of sIL-1RII are detectable in biological fluids under conditions of immunological disorders (Van Deuren et al., 1997; Juffermans et al., 1998; Laurincova, 2000). The released sIL-1RII keeps its ability to bind with high affinity to IL-1, particularly the circulating form (IL-1β), and to neutralize its biological effects
and MMP-9 (antibody to detect IL-1RII and MMP-9 respectively. Superimposing fluorescein (green) and rhodamine (red) shows co-expression of IL-1RII and MMP-9.

Figure 5. Dual immunofluorescent staining of IL-1RII and MMP-9. Dual immunofluorescent staining of IL-1RII (A and D) and MMP-9 (B and E) in the endometrial tissue of normal women (A, B and C) and women with endometriosis (D, E and F). Tissue sections were incubated with goat polyclonal anti-srhIL-1RII antibody and with mouse anti-hMMP-9 antibody. Sections were then incubated with rabbit anti-goat biotinylated antibody and then simultaneously with fluorescein isothiocyanate-conjugated streptavidin and rhodamine-conjugated sheep anti-mouse antibody to detect IL-1RII and MMP-9 respectively. Superimposing fluorescein (green) and rhodamine (red) shows co-expression of IL-1RII and MMP-9 (C and F). Scale bars = 20 mm.

(Colotta et al., 1994; Laurincova, 2000). Soluble IL-1RII appears to act by sequestering IL-1β within the extracellular compartment, thus restricting its availability and interaction with IL-1RI. In addition, sIL-1RII binds and blocks processing of IL-1β precursor, inhibits its maturation, loses affinity for IL-1 receptor antagonist (IL-1ra) and does not therefore interfere with IL-1ra-mediated inhibition of IL-1 effects (Dunne and O’Neill, 2003). Such a release of the soluble decoy receptor has been proposed to be an early event in the inflammatory cascade that acts to limit its severity. Therefore, the reduced release of sIL-1RII by the endometrial tissue of women with endometriosis indicates a deficiency in the capability of this tissue to down-regulate IL-1-mediated effects.

It is well documented that proteases such as MMP contribute to sIL-1RII shedding from the cell surface (Orlando et al., 1997; Cui et al., 2003). Numerous MMP were reported to be expressed in the normal human endometrial tissue, where they appear to play a significant role in normal tissue remodelling during the sequential phases of proliferation, differentiation and tissue breakdown during menstruation (Curry and Osteen, 2003). MMP may be therefore involved in the cleavage of the membrane-bound IL-1RII and normal shedding of sIL-1RII from endometrial cells. In endometriosis, several proteases including MMP were found to have an increased expression in ectopic and eutopic endometrial tissues and to be involved in the invasive establishment of the disease (Osteen et al., 2003; Liu et al., 2002; Gilabert Estelles et al., 2003). Our previous studies revealed an increased release of proteolytic activity by the eutopic endometrium in women with endometriosis as compared to normal women and identified MMP-9 as one of the overproduced proteases by this tissue (Collette et al., 2004). Data of the present study corroborated these findings and further showed a significant correlation between increased proteolysis and decreased sIL-1RII levels. A trend for a correlation between MMP-9 secretion and sIL-1RII concentrations was also noted. Furthermore, our data showed a marked degradation of [125I]srhIL-1RII in the culture medium of endometriosis women-derived endometrial tissue, which significantly correlated with the proteolytic activity and showed a tendency for a correlation with MMP-9 concentrations. Therefore, while proteases contribute to sIL-1RII shedding (Orlando et al., 1997; Cui et al., 2003), our data suggest that their elevated concentrations in the endometrium of endometriosis patients may amplify the degradation of the soluble receptor and further reduce its availability. Furthermore, TIMP-1, a natural specific inhibitor of several MMP including MMP-9, partially inhibited [125I]srhIL-1RII degradation. The degradation of [125I]srhIL-1RII was also inhibited, although less markedly, by anti-hMMP-9 antibody, which indicates that MMP may contribute to sIL-1RII instability and suggests a role for MMP-9. Incubation of srhIL-1RII with active rhMMP-9 resulted in a dose-dependent degradation of the soluble receptor as shown by western blot analysis, and preincubation of rhMMP-9 with anti-hMMP-9 antibody or rhTIMP-1 inhibited srhIL-1RII degradation. This is in agreement with the presence of at least two putative cleavage sites for MMP-9 in sIL-1RII amino acid sequence as reported previously (McMahan et al., 1991; Kridel et al., 2001). Moreover, dual immunofluorescence showed an increased immunostaining for MMP-9 in situ in the endometrial tissue of women with endometriosis compared to normal women and a decreased immunostaining for IL-1RII. It is not documented yet whether sIL-1RII and MMP-9 form part of the same complex. Proteases other than MMP were shown to be involved in sIL-1RII shedding (Cui et al., 2003). These proteases might also be implicated in sIL-1RII protein degradation since TIMP-1 alone was unable to completely block the degradation of [125I]rhsIL-1RII. Further studies will be needed to investigate sIL-1RII–MMP-9 interaction and the involvement of other possible proteases. Proteolysis-mediated
regulation of inflammation is a well-documented regulatory mechanism and described in a number of immuno-inflammatory disorders (Hiemstra, 2002). Several MMP were reported to cleave adhesion molecules, cytokines, chemokines, growth factors and binding proteins and to play an important role in positive or negative regulation of inflammation (Mohammed et al., 2003).

The present study reveals a post-translational mechanism by which proteases may contribute to down-regulating IL-1RII protein levels in the endometrial tissue of endometriosis women. Indeed, it is quite possible that decreased IL-1RII levels observed in the endometrial tissue of endometriosis patients (Kharfi et al., 2002) may by itself contribute to increased protease secretion as a result of an increased cell responsiveness to IL-1. It is well known that protease release by endometrial cells can be stimulated by IL-1 (Rawdanowicz et al., 1994) and that endometrial cells from endometriosis patients secrete more MMP or proteases in response to IL-1 (Sillem et al., 2001). Nevertheless, the fact that increased protease release accelerates sIL-1RII degradation makes this latter less available for binding to IL-1. This may further amplify IL-1-mediated cell activation and accentuate the immuno-inflammatory process observed locally in the eutopic endometrium of patients, as well as in the ectopic locations where this tissue can migrate, implant and develop into endometriosis lesions.

In conclusion, the present study showed for the first time a reduced release of sIL-1RII by the endometrial tissue of women with endometriosis and revealed a proteolytic post-translational mechanism which may be involved in the down-regulation of IL-1RII levels. Furthermore, it suggests that MMP and in particular MMP-9 may contribute to endometriosis-associated diminution of sIL-1RII stability. Such mechanisms may reduce the availability of the decoy sIL-1RII for IL-1 binding, thereby enhancing IL-1-mediated cell activation and accentuating the local immuno-inflammatory process observed in the eutopic endometrium of endometriosis patients.

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