Imbalance in the expression of the activating type I and the inhibitory type II interleukin 1 receptors in endometriosis

Ali Akoum1,2,3, C.Lawson1,2, C.Herrmann-Lavoie1,2 and R.Maheux1,2

1Unité d’Endocrinologie de la Reproduction, Centre de Recherche, Hôpital Saint-François d’Assise, Centre Hospitalier Universitaire de Québec, Canada and 2Département d’Obstétrique et Gynécologie, Faculté de Médecine, Université Laval, Québec, Canada
3To whom correspondence should be addressed at: Unité d’Endocrinologie de la Reproduction, Centre de Recherche, Hôpital Saint-François d’Assise, Centre Hospitalier Universitaire de Québec, 10 rue de l’Espinay, Local D0-711, Québec, Canada G1L 3L5. Tel: +1 418 525 4444 53329; Fax: +1 418 525 4195; E-mail: ali.akoum@crsfa.ulaval.ca

BACKGROUND: The ectopic establishment and progression of endometrial tissue is dependent upon its interaction with and responsiveness to the stimuli present in its new environment. Immune cell-derived cytokines, such as interleukin 1 (IL1), may alone or in concert with estrogens enhance the capability of ectopic endometrial cells to implant and develop into the host tissue. The objective of this study was to further evaluate the expression and significance of IL1 receptor type I (IL1R1), the signalling receptor that mediates cell activation by IL1, and IL1 receptor type II (IL1R2), a potent and specific down-regulator of IL1 action, in normal compared to endometriotic/endometrial tissues. METHODS: Techniques included immunohistochemistry, immunofluorescent staining, ELISA, western blotting and endometriotic cell culture transfection. RESULTS: Our study showed an imbalance in the expression of IL1R1 and IL1R2 in eutopic, and particularly in ectopic, endometrial tissues of women with endometriosis. Actually, a decreased IL1R2 expression is predominant in the eutopic and ectopic endometrium of women with endometriosis when compared with normal women, whereas a concomitant increase in IL1R1 expression occurs in ectopic endometrial tissue in comparison to eutopic, endometrial tissues of women with endometriosis. Actually, a decreased IL1R2 expression is predominant in the eutopic and ectopic endometrium of women with endometriosis when compared with normal women, whereas a concomitant increase in IL1R1 expression occurs in ectopic endometrial tissue in comparison to eutopic endometrial tissue of normal or endometriotic women, particularly in the initial and most active implants. Transfection of endometriotic cells with a cDNA coding for IL1R2 resulted in a significant decrease in IL1-induced secretion of vascular endothelial cell growth factor and monocyte chemotactic protein 1. CONCLUSIONS: IL1R1/IL1R2 imbalance may amplify endometrial cell responsiveness to IL1 and represent a key mechanism underlying the ability of these cells to implant and develop into host tissues.

Key words: endometriosis/endometrium/IL1R1/IL1R2/lesion

Introduction

Endometriosis is a frequent gynaecological disease affecting up to 10% of women of reproductive age and a major cause of abdominal pain, dysmenorrhoea, dyspareunia and infertility (Giudice, 2003). According to the most accepted theory, endometriosis (at least peritoneal endometriosis) is due to the tubal reflux of menstrual debris into the peritoneal cavity, which develops and grows into the peritoneum and the pelvic organs (Sampson, 1927). The etiology of endometriosis is still not clearly elucidated, but appears to involve a complex interplay of multiple genetic, environmental, hormonal and immunological factors (Lebovic et al., 2001b; Barlow and Kennedy, 2005; Ulukus and Arici, 2005). Endometriosis is associated with a chronic immunoinflammatory process that has been described locally in the peritoneal cavity where the disease often develops and the eutopic endometrium where it is believed to take origin (Jolicoeur et al., 1998; Sharpe-Timms, 2005; Ulukus and Arici, 2005).

Inflammation around and within active endometrial implants, and increased leukocyte infiltration and cytokine secretion in ectopic and eutopic endometrial tissues have widely been described (Ota et al., 1996; Hill et al., 1988; Jolicoeur et al., 1998; Jones et al., 1998; Witz, 2002; Sharpe-Timms, 2005; Ulukus and Arici, 2005).

The secretion of proinflammatory and mitogenic proteins by over-reactive endometrial cells in response to peritoneal stimuli following tubal reflux, and by endometriotic and associated immune cells, into the peritoneal environment may contribute to a cascade of events favouring tissue remodelling and invasion of the host tissue, angiogenesis and cell proliferation in the growing lesions, as well as further chemotraction of leukocytes to these foci of peritoneal inflammation.

Interleukin 1 (IL1), a principal macrophage-derived and major proinflammatory cytokine, may play a central role in the integrated inflammatory cascade associated with endometriosis and in propagating endometriotic implants through...
proinflammatory stimuli and synthesis of chemokines, growth factors and angiogenic factors (Akoum et al., 1995a, 1996b, 2001b; Lebovic et al., 2001a). In women with endometriosis, peripheral blood monocytes (Zeller et al., 1987), as well as peritoneal macrophages (Mori et al., 1992), were found to secrete elevated levels of IL1. Increased concentrations of the cytokine were found in the peritoneal fluid of women with endometriosis (Fakih et al., 1987; Mori et al., 1992; Taketani et al., 1992) as well as in ectopic endometrial implants (Bergqvist et al., 2001).

Our and other previous studies showed augmented sensitivity of ectopic endometrial cells to the biological actions of IL1 (Akoum et al., 1995a, 2001b, 1996b, 2002; Lebovic et al., 2000). These cells displayed increased secretion of monocyte chemotactic protein 1 (MCP1), Regulated on activation, normally T-cell expressed and secreted (Akoum et al., 2002) and angiogenic factors, such as vascular endothelial growth factor (VEGF), in response to IL1 (Lebovic et al., 2000). Increased cell responsiveness to IL1 was found in eutopic endometrial cells as well. These cells released higher amounts of MCP1 in vitro and showed enhanced integrin-mediated adhesion to extracellular matrix proteins (Silleh et al., 1999) in response to IL1, when compared with cells from normal women (Akoum et al., 1995a,b; Jolicoeur et al., 1998). In keeping with our findings, data from Lebovic et al. (2000) suggest that the ability of IL1B to activate an angiogenic phenotype in endometriotic stromal cells, but not in stromal cells from normal endometrium, is mediated by the functional signalling IL1 receptor type I (IL1R1).

IL1 has two known receptors, now designated as IL1R1 and IL1R2, and one receptor antagonist (IL1RA) which competes with IL1 for binding to IL1R1. Cell activation by IL1 results from its binding to cell surface IL1R1 which in concert with IL1R accessory protein (IL1RAP) is capable of transducing the activation signal (Dinarello, 2004). The cytokine’s receptor type II (IL1R2) has, in contrast to IL1R1, no signalling properties, but has been described as a potent, specific and natural inhibitor of IL1. This decoy receptor acts by sequestrating active and inactive IL1, thereby restricting the availability of the ligand for the functional receptor and inhibiting even its maturation (Colotta et al., 1994; Bossu et al., 1995; Symons et al., 1995; Subramaniam et al., 2004).

Our previous studies showed a significant decrease in IL1R2 expression in the eutopic endometrial tissue of women suffering from endometriosis when compared with healthy women (Akoum et al., 2001a). The present study points to a more profound defect in endometrial and endometriotic cell receptivity to IL1, as it reveals an imbalance in IL1R1 and IL1R2 expression occurring in the eutopic endometrium of women with endometriosis and more markedly in the ectopic endometrial tissue. Actually, although a decreased IL1R2 expression is predominant in the eutopic endometrium, a concomitant increase in IL1R1 expression occurs in ectopic endometrial tissue, particularly in the initial and most active implants. Overexpression of the activating IL1R1 combined with depression of the decoy IL1R2 may further amplify endometrial cell responsiveness to IL1 in the ectopic sites and represent a key mechanism underlying the ability of these cells to implant and develop into host tissues.

**Materials and methods**

**Source and handling of tissue**

Women who were recruited into the study had provided informed consent for a research protocol approved by Saint-François d’Assise Hospital Ethics Committee on Human Research. Women included in the study had no signs of endometrial hyperplasia or neoplasia and were not receiving any anti-inflammatory or hormonal medication during a period of at least 3 months before surgery. Endometriosis was diagnosed during investigative laparoscopy for infertility and/or pelvic pain or during a tubal ligation. Women had no other pelvic pathology, and the disease’s stage was determined according to the Revised American Fertility Society (1997) classification system. Patients with endometriosis (n = 25) had a mean age of 32.5 ± 5.7 years. Thirteen had endometriosis stage I, eight had endometriosis stage II and four had endometriosis stage IV. Ten of the endometriotic tissues were from red lesions, nine were from typical blue lesions and six were from white lesions. Normal women (n = 27) had a mean age of 35.8 ± 5.1 years. They were fertile, requesting tubal ligation, having no visible evidence of endometriosis at laparoscopy. Menstrual cycle dating was determined by the cycle history and confirmed by histological dating of the endometrium (Noyes et al., 1975). Six controls were in the proliferative phase of the cycle and 21 were in the secretory phase. Among women with endometriosis, 7 were in the proliferative phase and 18 were in the secretory phase of the menstrual cycle.

Matched endometriotic and endometrial biopsies from women with endometriosis and endometrial tissue from normal controls were obtained during laparoscopy. Tissues were immediately placed at 4°C in sterile Hank’s balanced salt solution (HBSS) (GIBCO Invitrogen Corp., Burlington, ON, Canada) containing 100 U ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin and 0.25 µg ml⁻¹ amphotericin and transported to the laboratory. Tissues were washed in HBSS at 4°C, snap frozen on dry ice in eppendorf tubes for western blot and ELISA or embedded in Tissue-Tek Optimal Cutting Temperature (OCT) compound (Miles, Inc., Elkhart, IN, USA) and stored at −80°C for immunohistochemical studies. Some of the endometriotic specimens were fixed in Tissufix #2 solution (Laboratoire Gilles Chaput Inc., Montreal, Quebec, Canada) for 24 h. They were then placed in a Tissue-Tek VIP apparatus (Miles Scientific, Etobicoke, ON, Canada) for another 12 h before being embedded in paraffin (TissuePrep, Fisher Scientific, Fair Lawn, NJ, USA) for pathological investigations.

**Immunohistochemistry**

IL1R1 immunostaining was performed using a polyclonal rabbit anti-human IL1R1 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) [10 µg ml⁻¹ in phosphate-buffered saline (PBS)/0.2% bovine serum albumin (BSA)], a biotin-conjugated goat anti-rabbit IgG (H + L) (Jackson Immuno Research Laboratories Inc., West Grove, PA, USA) (1 : 1000 dilution in PBS/0.2%BSA/0.1%Tween 20), peroxidase-conjugated streptavidin (Jackson ImmunoResearch Laboratories Inc.) (1 : 500 dilution in PBS/0.2%BSA/0.1%Tween 20) conjugated to horseradish peroxidase.
20) and 3,3′-diaminobenzidine (DAB) (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada). Haematoxylin was used for counterstaining.

IL1R2 immunostaining was performed as described previously (Akoum et al., 2001a). Sections incubated with an equivalent concentration of normal rabbit or mouse IgGs instead of the polyclonal rabbit anti-human IL1R1 or the mouse monoclonal anti-human IL1R2 antibody, respectively, were included as negative controls in all experiments. Slides were viewed using a microscope (Leica mikroskopie und systeme GmbH, model DMRB, Leica Corp., Postfach, Wetzlar, Germany) connected to an image analysis system (ISIS, Metasystems, Altlussheim, Germany).

**Immunofluorescent staining**

Briefly, tissue sections or endometriotic cell cultures in Lab-Tek 8-chamber slides (Nalge Nunc International, Naperville, IL, USA) were successively incubated with rabbit polyclonal anti-human IL1R1 antibody, mouse monoclonal anti-human IL1R2 antibody and biotin-conjugated goat anti-rabbit IgG, according to the conditions described earlier (immunohistochemistry section). They were then incubated with Alexa 488-labelled streptavidine (1 : 100 dilution in PBS/0.2% BSA) and Alexa Fluor 594-labelled goat anti-mouse antibody (1 : 1000 dilution in PBS/0.2% BSA) (Molecular Probes Inc., Eugene, OR, USA) simultaneously. 4',6-diamidino-2-phenyl-indole (DAPI) (1 : 2000 dilution in PBS/0.1% Tween 20) was used for counterstaining. Sections were observed under the Leica microscope mentioned above.

**IL1R1 and IL1R2 ELISA**

IL1R2 concentrations in total proteins extracted from frozen endometrial tissues (Bigonnesse et al., 2001) or in culture supernatants were measured using our previously reported procedure (Bellehumeur et al., 2005). IL1R1 concentrations were measured according to a similar procedure, but with the use of a mouse monoclonal anti-human IL1R1 antibody for coating (500 ng per well in PBS/0.5% BSA) and a goat polyclonal anti-human IL1R1 antibody (1 μg ml⁻¹ in PBS/0.5% BSA) (R&D Systems) for detection. IL1R1 and IL1R2 concentrations were calculated by interpolation from standard curves.

**Western blotting**

Protein extraction, SDS–polyacrylamide gel electrophoresis and transfer onto nitrocellulose membranes were performed as we reported previously (Akoum et al., 2001a). Briefly, a goat polyclonal anti-human IL1R1 (2 μg ml⁻¹ in PBS containing 1% BSA and 0.1% Tween 20) or a goat polyclonal anti-human IL1R2 antibody (R&D Systems) (2 μg ml⁻¹ in PBS/BSA/Tween 20) were used for detection, followed by Fc-specific peroxidase-labelled rabbit anti-goat antibody (Jackson ImmunoResearch Laboratories Inc.) (1 : 10000 dilution in PBS/BSA/Tween 20) and enhanced chemiluminescence system (BM chemiluminescence blotting substrate, POD) (Roche Diagnostics, Laval, QC, Canada), respectively. Membranes were exposed to Biomax film (Eastman Kodak, Rochester, NY, USA). Controls included recombinant human-soluble (rhs) IL1R1 and IL1R2 (R&D Systems), used as positive controls, incubation with equivalent concentrations of normal goat IgGs and pre-absorption of the primary anti-IL1R1 and anti-IL1R2 antibodies with 5 μg ml⁻¹ (0.09 μM) of rhsIL1R1 and 5 μg ml⁻¹ (0.11 μM) of rhsIL1R2, respectively. Membranes were rebotted with an anti-α-actin antibody (1/1000 dilution in PBS/BSA/Tween 20) (Iowa University, developmental studies hybridoma bank) to ensure equal protein loading.

**Endometriotic cell culture and transfection**

Endometriotic tissue was minced into small pieces and dissociated with collagenase as previously described (Akoum et al., 1995a). Cells were pelleted by centrifugation (200 g/10 min) and plated in Dulbecco’s modified Eagle’s medium (DMEM)-F12 medium containing 10 μg ml⁻¹ insulin, 5 μg ml⁻¹ transferrin and 10% fetal bovine serum (FBS) at 37°C, 5% carbon dioxide. In this study, no attempt was made to separate epithelial and stromal fibroblast-like cells. These cells were identified morphologically in culture by light microscopy and immunocytochemically with specific monoclonal antibodies to cytokeratins and vimentin as previously described (Akoum et al., 1995a). No leukocytes were detected in the endometriotic cells detached from culture dishes and assessed by fluorescence-activated cell sorting (data not shown). Cells were transfected with the eukaryotic expression vector pcDNA3 either alone or containing a cDNA coding for IL1R2 (Bassou et al., 1995). Transfection was performed using Lipofectamine Plus reagent according to manufacturer’s instructions (Invitrogen, Burlington, Ontario, Canada). Endometriotic cells were used after one passage. Tadpole-shaped glandular epithelial cells were present in our endometriotic cell cultures, but cultures appeared morphologically to contain more stromal than epithelial cells. Cells were seeded in six-well culture plates (Costar, Cambridge, MA, USA), cultured in DMEM-F12 medium containing 10% FBS and 1% antibiotics (Invitrogen) until 70% confluence and incubated with DNA/Lipofectamine complexes in FBS-free DMEM medium. After 3 h of incubation at 37°C, medium containing 10% FBS was added and culture pursued for 48 h. Before cell stimulation, the culture medium was replaced by a serum-free medium for 24 h. Cells were then exposed or not for 6 h to IL1B (0–1 ng ml⁻¹) (Invitrogen) diluted in a fresh FBS-free medium. The culture supernatants were collected and kept in small aliquots at −80°C until use by ELISA. Cells were recovered in a cold buffer solution containing 0.5% Triton X-100, 10 mM HEPES (pH 7.4), 150 mM NaCl, 2 mM ethylene glycol tetra-acetic acid, 2 mM ethylene diamine tetra-acetic acid and 0.02% Na3 and a mixture of anti-proteases composed of 5 μM aprotinin, 63 μM leupeptin and 3 mM phenylmethylsulphonylfluoride and kept in small aliquots at −80°C.

**VEGF and MCP1 ELISA**

VEGF ELISA was performed using a Human VEGF CytosetTM Kit (CHG0113, Biosource, Camarillo, CA, USA). The optical density was measured at 450 nm and VEGF concentrations were extrapolated from a standard curve. The sensitivity of the assay was ~20 pg ml⁻¹. MCP1 ELISA was carried out according to our previously described procedures.
(Akoum et al., 1996a). The sensitivity of the assay was approximately 10 pg ml$^{-1}$.

**Statistical analysis**

IL1R1 concentrations in tissue protein extracts and sIL1R2, VEGF and MCP1 concentrations in the culture supernatants followed a parametric distribution and were therefore statistically analysed using one-way analysis of variance (ANOVA) and the Bonferroni’s post hoc test for multiple comparisons or the unpaired $t$-test for comparison of two groups. The paired $t$-test was used to compare IL1R1 data from endometrial and matched endometriotic tissues. IL1R2 concentrations in tissue protein extracts followed a non-parametric distribution and were statistically analysed using the Kruskal–Wallis test and the Dunn’s test *post hoc* for multiple comparisons or the Mann–Whitney test for comparison of two groups. The Wilcoxon matched pairs test was used to compare IL1R2 data from endometrial and matched endometriotic tissues. All analyses were performed using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, USA). Differences were considered as statistically significant for $P < 0.05$.

**Results**

In normal women, immunoreactive IL1R1 and IL1R2 were detectable throughout endometrial tissues, but were more prominent in endometrial glands (Figure 1A and B, respectively). In women with endometriosis, IL1R1 showed a similar pattern of immunostaining, which, nevertheless, appeared to be more intense in endometriotic tissue (Figure 1G) when compared with endometrial tissue from normal women (Figure 1A) or matched endometrial tissue from women with endometriosis (Figure 1D). However, IL1R2 immunostaining was noticeably reduced in endometriotic tissue (Figure 1H). No immunostaining was observed in serial sections incubated with an equivalent concentration of mouse (C) or rabbit (F and I) IgGs instead of the primary mouse anti-IL1R2 antibody or the rabbit anti-IL1R1 antibody, respectively (negative controls). Scale bar: 30 μm.

**Figure 1.** Interleukin 1 receptor types I and II (IL1R1 and IL1R2) immunostaining in ectopic and eutopic endometrial tissues. Sections of endometrial tissue from normal women (A and B) and matched endometrial (D and E) and endometriotic (G and H) tissues from women with endometriosis were examined for IL1R1 (A, D and G) or IL1R2 (B, E and H) expression (brown staining). Haematoxylin was used for counterstaining (blue staining). In this figure, tissues were from a normal woman and a woman with endometriosis at days 26 and 24 of the menstrual cycle, respectively. Note the intense immunostaining of IL1R1 in normal (A) and endometriosis (D) women-derived endometrial tissues and particularly in endometriotic tissue (G). Note the intense immunostaining for IL1R2 in a serial section from the endometrial tissue of normal woman (B) and the reduced intensity of IL1R2 immunostaining in serial sections from the endometrial (E) and endometriotic (H) tissues of endometriosis patient. No immunostaining was observed in serial sections incubated with an equivalent concentration of mouse (C) or rabbit (F and I) IgGs instead of the primary mouse anti-IL1R2 antibody or the rabbit anti-IL1R1 antibody, respectively (negative controls). Scale bar: 30 μm.
less intense in endometrial (Figure 1E) and endometriotic (Figure 1H) tissues from women with endometriosis, when compared with endometrial tissue from normal women (Figure 1B). Incubation of tissue sections with normal rabbit or mouse IgGs used at concentrations equivalent to that of the primary rabbit polyclonal anti-human IL1R1 antibody or the mouse monoclonal anti-human IL1R2 antibody, respectively (negative controls), did not result in any noticeable staining. Examples of negative controls are shown in Figure 1C, F and I.

To further examine IL1R1 and IL1R2 expression in situ in the endometrial and the endometriotic tissues, simultaneous immunofluorescent staining of the receptors was performed. Data illustrated in Figure 2 confirmed the above observations and showed a green fluorescence, corresponding to IL1R1, which was more intense in endometriotic tissue (Figure 2G) than in normal (Figure 2A) and matched endometriosis (Figure 2D) women-derived endometrial tissues. In contrast, the red fluorescence, corresponding to IL1R2, was markedly less intense in endometriotic (Figure 2H) and matched endometrial (Figure 2E) tissues from women with endometriosis than in endometrial tissue from normal women (Figure 2B). Merged pictures of IL1R1 and IL1R2 staining clearly show a predominant green fluorescence in endometriosis women-derived endometrial (Figure 2F) and endometriotic (Figure 2I) tissues, when compared with normal women-derived endometrial tissue (Figure 2C) where a yellow colour corresponding to simultaneous expression of IL1R1 and IL1R2 could be seen. These immunohistochemical studies have been repeated and confirmed in tissues from five normal women and seven women with endometriosis.

IL1R1 and IL1R2 levels in endometrial and endometriotic tissues were then measured by ELISA. As shown in Figure 3A, IL1R1 concentrations were significantly higher in endometriotic tissue than in endometrial tissue from normal (P < 0.01) or endometriosis (P < 0.05) women, whereas no statistically significant difference between endometrial tissues from normal and endometriosis women was seen (P = 0.28). On the other hand, IL1R2 concentrations in endometriotic and endometrial tissues of women with endometriosis were significantly lower than that in endometrial tissue of normal women. 

Figure 2. Simultaneous immunofluorescent staining of IL1R1 and IL1R2 in ectopic and eutopic endometrial tissues. IL11R1 and IL1R2 were immunostained as described in Materials and methods, and DAPI (blue) was used for counterstaining. In this figure, tissues were from a normal woman and a woman with endometriosis at day 26 of the menstrual cycle. Note the green fluorescence corresponding to IL1R1 which is intense in normal (A) and endometriosis (D) women-derived endometrial tissues and particularly in matched endometriotic tissue (G). The red fluorescence corresponding to IL1R2 is markedly less intense in endometriotic (H) and matched endometrial (E) tissues from women with endometriosis than in endometrial tissue from normal women (B). Superposition of the green and the red signals shows simultaneous immunostaining (yellow) of IL1R1 and IL1R2. Note the predominant green fluorescence in endometriosis women-derived endometrial (F) and endometriotic (I) tissues, when compared with normal women-derived endometrial tissue (C) where a yellow colour corresponding to simultaneous expression of IL1R1 and IL1R2 expression could be seen. Scale bars: 30 μm.
women (P < 0.01 and P < 0.05, respectively), but no statistically significant difference between endometriotic and endometrial tissues of women with endometriosis was noted (P = 0.12) (Figure 3B).

Interestingly, analysis of the data according to the type of endometriotic lesion further revealed a marked increase in IL1R1 in active red endometriotic lesions when compared with endometrial tissue from normal (P < 0.001) and endometriosis (P < 0.001) women. In the typical black–blue lesions, IL1R1 levels were significantly higher than in endometrial tissue from normal women (P < 0.01), whereas no statistically significant difference between white endometriotic lesions and endometrial tissue from normal or endometriosis women was found (P = 0.76 and P = 0.72, respectively) (Figure 3A). Interestingly, IL1R2 levels were significantly reduced in red (P < 0.001) and typical (P < 0.01) endometriotic lesions when compared with normal women-derived control endometrial tissue, whereas no statistically significant change in IL1R2 levels in white endometriotic lesions was noted (P = 0.27) (Figure 3B).

Analysis of IL1 receptors’ expression according to the menstrual cycle phase showed that IL1R1 concentrations in endometriotic tissue were significantly higher than that in endometrial tissue of normal women, both in the proliferative (P < 0.05) and the secretory (P < 0.05) phases of the cycle. The difference in IL1R1 concentrations between endometriotic and endometrial tissues from women with endometriosis was significant in the proliferative phase of the menstrual cycle (P < 0.05), but did not reach the level of statistical significance in the secretory phase (P = 0.13). IL1R2 concentrations in endometriotic tissue were significantly lower than that in endometrial tissue of normal women in the proliferative phase of the cycle (P < 0.05), but a more marked difference was noted in the secretory phase (P < 0.001). IL1R2 concentrations in the endometrial tissue of women with endometriosis were also significantly lower than in normal women in the proliferative (P < 0.05) and the secretory (P < 0.05) phases of the menstrual cycle. Otherwise, no statistically significant difference in IL1R2 levels between endometriotic and endometrial tissues from women with endometriosis in the proliferative or the secretory phases of the menstrual cycle was observed (P = 0.49 and P = 0.21, respectively).

Both IL1R1 and IL1R2 can be released from the cell surface following proteolytic cleavage (Orlando et al., 1997; Cui et al., 2003). Therefore, we examined which of soluble (s) or membrane-bound (mb) IL1R1 and IL1R2 forms varied in endometrial and endometriotic tissues of women with endometriosis. Western blot analysis of IL1R1 showed a faint band whose apparent molecular weight (MW) corresponds to mbIL1R1 (90 kDa), an intense band corresponding to sIL1R1 (55 kDa) and two other bands of 73.3 and 69 kDa which may correspond to degraded IL1R1 protein. Pre-absorption of the anti-IL1R1 antibody with an excess of rhsIL1R1 before incubation with blotted proteins resulted in a noticeable attenuation of the intensity of these bands, thereby demonstrating specific binding (Figure 4A). Western blot analysis of IL1R2 showed a 68 kDa band and a doublet of 45 and 48 kDa MW bands. The 68 and 45 kDa bands correspond to the reported MWs of mbIL1R2 and sIL1R2, respectively (Boraschi et al., 1996), and the doublet, reported previously (Akoum et al., 2001a),

Figure 3. Graphical illustration of IL1R1 and IL1R2 levels. IL1R1 (A) and IL1R2 (B) concentrations (ng mg⁻¹ of total proteins) were measured by ELISA in normal endometrial tissues (NE, n = 27) and in endometrial (EE, n = 25) and endometriotic (ET, n = 25) tissues of patients with endometriosis. Endometriotic tissues were also grouped according to the lesion’s type (ET red, n = 10; ET typical, n = 9; ET white, n = 6). Results were expressed as means ± SEM. *P < 0.05, **P < 0.01 and ***P < 0.001, respectively, when compared with NE. †P < 0.05 and ‡P < 0.001, respectively when compared with EE.

Figure 4. Western blot analysis of IL1R1 and IL1R2 expression in the endometrial tissue. For IL1R1 (A), western blotting detected a 90 kDa band, which corresponds to the membrane-bound (mb) form of IL1R1, a 55 kDa band corresponding to the soluble (s) form of the receptor and two other bands of 73.3 and 69 kDa which may correspond to degraded IL1R1 (lane 1). Controls included rhsIL1R1 protein (lane 2) and pre-absorption of the anti-IL1R1 antibody with an excess of rhsIL1R1 (lane 3). For IL1R2 (B), western blotting detected a 68 kDa band, which corresponds to mbIL1R2, and a doublet of 45 and 48 kDa MW corresponding to sIL1R2 (lane 2). Controls included rhsIL1R2 protein (lane 1) and pre-absorption of the anti-IL1R2 antibody with an excess of rhsIL1R2 (lane 3). In this figure, tissues were from normal women at cycle days 22 (A) and 15 (B).
may correspond to two forms of sIL1R2. Pre-absorption of the anti-IL1R2 antibody with an excess of rhsIL1R2 before incubation with blotted proteins resulted in a noticeable diminution of the intensity of these bands, thereby demonstrating specific binding (Figure 4B). Furthermore, for the same amount of total endometrial proteins, as shown by the intensity of the corresponding α-actin bands, both mbIL1R1 and sIL1R1 bands were more intense in endometriotic lesions when compared with endometrial tissues from normal and endometriosis women, whereas mbIL1R2 and sIL1R2 bands were markedly less intense in endometriotic and endometrial tissues of women with endometriosis when compared with endometrial tissues from normal women (Figure 5).

Ectopic endometrial cells were then cultured and assessed for VEGF and MCP-1 secretion in response to IL1, before and after transfection with the pcDNA3 expression vector containing IL1R2 cDNA or the empty pcDNA3 vector. Dual immunocytofluorescence analysis showed a faint immunostaining for IL1R2 in endometriotic cells which were not transfected or transfected with the empty pcDNA3 vector and a marked immunostaining for this receptor in IL1R2 cDNA-transfected cells (Figure 6B). IL1R1 immunostaining was intense in non-transfected endometriotic cells and showed no change following transfection with IL1R2 cDNA or the pcDNA3 vector alone (Figure 6A). Cell stimulation with IL1β showed a significant dose-dependent increase in VEGF and MCP1 secretion by non-transfected endometriotic cells, and showed no marked decrease in endometriotic and endometrial tissues of women, whereas the expression of the signalling activating IL1R1 was co-amplified by a more marked decrease in the expression of IL1R2 and a concomitant increase in the expression of IL1R1.

These findings may have an interesting significance. They first suggest that the eutopic endometrial tissue of women with endometriosis is intrinsically less capable of buffering or counterbalancing IL1 effects and is more prone to respond to this major proinflammatory and multifunctional cytokine in the ectopic sites following retrograde menstruation. Second, the endometrial tissue that has had the capability to implant in ectopic sites is even more responsive or sensitive to IL1 than the eutopic endometrial tissue because of the simultaneous increase in IL1R1 expression and the more pronounced decrease in the expression of IL1R2.

The mechanisms underlying such an accentuated imbalance in the ectopic endometrial tissue remain unknown. One could hypothesize, however, that it might be due to the successful selection of highly cytokine-sensitive endometrial cells displaced into the peritoneal cavity by retrograde menstruation. It is still possible also that local yet unidentified peritoneal factors will up-regulate IL1R1 expression in ectopic endometrial cells and further down-regulate the expression of IL1R2. Little is known about the regulation of IL1 receptors. IL1 is known for up-regulating its own receptor type I in endometrial cells (Simon et al., 1994), but its effects on the receptor type II remain unknown. Chemoattractants, such as IL8, and proinflammatory cytokines, such as tumor necrosis factor alpha, were shown to induce IL1R2 shedding from the cell surface (Colotta et al., 1995; Orlando et al., 1997), and according to our studies (Bellehumeur et al., 2005), proteases such as matrix metalloproteinases, which mediate IL1R2 shedding (Orlando et al., 1997; Cui et al., 2003), can increase sIL1R2 degradation. These factors, whose peritoneal concentrations were shown to be higher in endometriosis patients (Szamatowicz et al., 2002; Huang et al., 2004; Ulukus and Arici, 2005), might therefore play a role in IL1R1/IL1R2 imbalance in the ectopic endometrium, but further studies are required to elucidate this.

Of further interest was the finding that IL1R1 and IL1R2 expression varied according to the type of endometriotic

![Figure 5. Western blot analysis of IL1R1 and IL1R2 expression in ectopic and eutopic endometrial tissues. (A) Strips were incubated with a goat polyclonal anti-human IL1R1 antibody. (B) Strips were incubated with a goat polyclonal anti-human IL1R2 antibody. Lane 1, endometrium from a normal woman (cycle day 8); lane 2 and 3, endometrium and endometriotic tissues from a woman with endometriosis (cycle day 7); lane 4, rhsIL1R1 (A) and rhsIL1R2 (B). α-Actin bands demonstrated equal protein loading.](https://academic.oup.com/humrep/article-abstract/22/5/1464/2914917)
lesions. In fact, although significant down-regulation of IL1R2 expression and up-regulation of that of IL1R1 were observed in red and typical endometriotic lesions, the most significant change in IL1R1 expression occurred in the red ones. Interestingly, red endometriotic lesions, having a high degree of vascularization and mitotic index, are believed to correspond to the first, active stage of early endometrial tissue implantation (Wiegerinck et al., 1993; Kokorine et al., 1997; Nisolle et al., 1997). Therefore, such an imbalance in the levels of the activating and the inhibitory receptors of IL1, mainly occurring in early, highly vascularized and active endometriotic lesions, is suggestive of a higher cell sensitivity for IL1 in these lesions and points to a process of cell activation through IL1 signalling that takes place in the ectopic endometrial tissue, particularly in its earliest forms. Furthermore, these findings are suggestive of a relationship between abnormal IL1 receptors’ expression and the implant’s activity.

Evidence available to date supports a central role for activated immune cells in the progression of endometriotic implants and offers an immunological explanation for the neovascularization that surrounds active endometriotic lesions (Taylor and Mueller, 2004). Macrophages from women with endometriosis were found to secrete elevated levels of IL1 (Zeller et al., 1987). Elevated concentrations of IL1 were found in the peritoneal fluid of women suffering from endometriosis (Fakih et al., 1987; Mori et al., 1992; Taketani et al., 1992). IL1 was shown to up-regulate the secretion of many cytokines and chemokines in endometriotic cells (Akoum et al., 1995a, 1996b, 2001b, 2002), to display more pronounced effects on endometrial cell adhesion in endometriosis (Sillem et al., 1999) and to enhance the production of angiogenic molecules such as VEGF in endometriotic cells, when compared with endometrial cells from normal endometrium (Lebovic et al., 2000). Interestingly, incubation of endometriotic cells with IL1B induced a dose-dependent increase in the secretion of VEGF and MCP1. IL1R2 was, in contrast to ILR1, not detected in these cells or in their culture medium. However, induction of IL1R2 expression by transfecting endometriotic cells with IL1R2 cDNA resulted in a significant down-regulation of VEGF and MCP1 secretion.

In view of these data and the wide spectrum of IL1 biological effects, a marked imbalance in IL1 receptors’...
expression in eutopic, ectopic and particularly in active and early endometriosis lesions may play an important role in promoting a successful implantation of endometrial tissue in the earliest steps of endometriosis following retrograde menstruation.

Although significant changes in IL1R1 and IL1R2 expression in endometriotic tissue were observed in the proliferative phase of the menstrual cycle, IL1R1/IL1R2 imbalance was more obvious in the secretory phase. This may further decrease the ability of this tissue to buffer IL1 effects, thus enhancing IL1-mediated cell activation and leading to an exaggerated peritoneal inflammatory response. However, the reasons of these menstrual cycle-dependent variations and the possible influence of the menstrual cycle steroids remain unknown.

The present study included matched endometrial and endometriotic tissues from only 21 stages I–II and 4 stages III–IV endometriosis women, which does not allow accurate assessment of the influence of endometriosis stage. Nevertheless, the decrease in IL1R2 expression observed in endometriotic tissue when compared with endometrial tissues from normal women and the simultaneous increase in the expression of IL1R1 were found to be statistically significant in endometriosis stages I–II \( (P < 0.01 \) and \( P < 0.001 \), respectively) and III–IV \( (P < 0.05 \) and \( P < 0.05 \), respectively).

Many levels of control for IL1 activity have been described. This is not surprising in view of the wide variety of biological properties displayed by this cytokine (Mantovani et al., 1996; Dinarello, 2004). Indeed, the involvement of other IL1 specific inhibitors in endometriosis such as IL1RA warrants further investigations. IL1RA acts by competing with IL1 for binding to IL1R1 (Dinarello, 2004). IL1RA expression was found to be absent in ectopic endometrial tissue, but no study had yet addressed its expression in endometrial and matched endometriotic tissues of women with endometriosis in comparison with normal women.

In conclusion, this study is the first to reveal an endometriosis-associated disequilibrium in IL1 control in eutopic endometrial cells, i.e. before these cells migrate and develop into endometriosis lesions, but more markedly in ectopic endometrial cells, particularly in the initial and most active endometrial implants. These findings suggest that endometrial cells of women with endometriosis not only have a reduced capability of neutralizing and counterbalancing IL1 effects due to decreased IL1R2 expression, but, by overexpressing IL1R1 in the ectopic sites, they are even more receptive/sensitive to this major proinflammatory cytokine. In view of the well-documented and significant role of IL1 in endometriosis pathophysiology and the wide variety of its biological properties including proangiogenic, growth and inflammatory effects, overexpression of the activating IL1R1 combined with a depression of the decoy IL1R2 may further amplify endometrial cell responsiveness to IL1 and represent a key mechanism underlying the ability of these cells to implant and develop into host tissues.

Acknowledgements

This work was supported by grant MOP-14638 to A.A. from The Canadian Institutes for Health Research. A.A. is Chercheur National from the Fonds de la Recherche en Santé du Québec (FRSQ). The authors wish to thank Dr Paola Bossù (Dompé SpA, L’Aquila, Italy), for providing the IL1R2-pcDNA3 plasmid, Drs Sylvie Bazin, François Bellumeur, Jacques Bergeron, Jean Blanchet, Pierre Blanchet, Bruno Camiré, Simon Carrier, Elphege Cyr, Jean-Yves Fontaine, Dre Julie Guillemette, Mathieu Leboeuf, Jacques Mailloux, Marie-Christine Roy, Jean-Pierre Verreault and Júlia Villa for patient evaluation and providing endometrial tissue samples, Nathalie Bourcier, Madeleine Desaulniers, Johanne Pelletier, Sylvia Pleau and Marie-Joséé Therriault for technical assistance and Dr Mahera Al-Akoum for statistical analyses.
References


Submitted on December 18, 2006; resubmitted on January 10, 2007; accepted on January 15, 2007