Tumour necrosis factor-α up-regulates macrophage migration inhibitory factor expression in endometrial stromal cells via the nuclear transcription factor NF-κB

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BACKGROUND: A series of controlled changes including proliferation, secretion and menstrual shedding occur in the human endometrium during every normal menstrual cycle. Macrophage migration inhibitory factor (MIF), a multifunctional cytokine with numerous proinflammatory, immunomodulatory and angiogenic properties, appears to be expressed in the human endometrium and to follow a regulated cycle phase-dependent expression, but the mechanisms underlying endometrial MIF expression remain to be fully elucidated. METHODS AND RESULTS: Results from enzyme-linked immunosorbent assay (ELISA) demonstrated a significant dose- and time-dependent increase in MIF secretion by human endometrial cells in response to tumour necrosis factor-alpha (TNF-α) (0.1–100 ng/ml). This increase was also observed at the mRNA level as shown by reverse transcription (RT)–PCR. Curcumin (10⁻⁸ mol/l), a known nuclear factor (NF)-κB inhibitor, inhibited the TNF-α-induced pIκB phosphorylation as shown by western blotting, NF-κB translocation into the nucleus as shown by electrophoretic mobility shift assay, and MIF synthesis and secretion as measured by ELISA and RT–PCR. The expression of a dominant-negative NF-κB inhibitor (IkB) significantly decreased the TNF-α-induced MIF promoter activity as analysed by transient cell transfection. CONCLUSIONS: These results indicate clearly that TNF-α up-regulates the expression of MIF in endometrial stromal cells. This took place possibly through NF-κB activation, and may play an important role in the physiology of the human endometrium.

Key words: endometrium/MIF/NF-κB/TNF-α

Introduction

Human endometrium undergoes a series of controlled changes including proliferation, secretion and menstrual shedding during every normal menstrual cycle. These dynamic processes require a network of hormonal, growth and immunoinflammatory factors in which cytokines locally produced within the endometrial tissue play a crucial role (Tabibzadeh, 1996; 1998; Lebovic et al., 2000; von Wolff et al., 2000; Bigonnesse et al., 2001; Boucher et al., 2001; Critchley et al., 2001). Of particular interest is the role that appears to be played by tumour necrosis factor-alpha (TNF-α) in human endometrial physiology.

TNF-α was first identified as a cytokine secreted by endotoxin-activated macrophages that induced the necrosis of tumours (Carswell et al., 1975). TNF-α is now known as a pluripotent cell mediator and angiogenic cytokine that promotes the production of other cytokines in various cells (Feldmann and Maini, 1999). The human endometrium is characterized by a variety of cell types, including fibroblasts, immune cells, vascular cells and epithelial cells, all of which express TNF-α (Hunt et al., 1992; Philippeaux and Piguet, 1993; Tabibzadeh et al., 1995; Chegini et al., 1999; von Wolff et al., 1999; Bergqvist et al., 2000; 2001). Despite discrepancies in the cycle expression patterns, these studies suggest a local role for TNF-α in a variety of normal endometrial functions. Increased expression of this cytokine was shown to cause pathophysiological effects reflected by its involvement in implantation failure (Hazout, 1995), abortion (Giacomucci et al., 1994) and endometriosis (Zhang et al., 1993).

Our previous studies identified macrophage migration inhibitory factor (MIF) as a potent mitogenic factor for human endothelial cells released by ectopic endometrial cells (Yang et al., 2000) in women with endometriosis (Kats et al., 2002b). Subsequent studies from our laboratory showed a cycle phase-dependent expression of MIF during the menstrual cycle, with a particular increase occurring in the late secretory phase, suggesting a tight regulation and perhaps different roles for this factor in the reparative, reproductive and inflammatory-like processes that occur in human endometrium during the normal
Materials and methods

Subjects and tissue handling

Endometrial biopsies were obtained during laparoscopy for tubal ligation from eight normal fertile women who had not received hormonal or anti-inflammatory therapy for at least 3 months before surgery (mean age ± SD, 30.71 ± 4.03 years). Four women were in the proliferative phase of the menstrual and four in the secretory phase. Written informed consent was obtained from these women under a study protocol approved by the Ethical Committee on Human Research at Laval University, Quebec, Canada. Biopsies were obtained by aspiration with the use of a Pipelle (Unimar Inc., Prodimed, Neuilly-En-Teuchelle, France), immediately placed at 4°C in sterile Hank’s balanced salt solution containing 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B (Invitrogen Life Technologies, Burlington, ON, Canada) and transported to the laboratory.

Cell culture and stimulation

Endometrial stromal cells were obtained and characterized according to our previously described procedure (Boucher et al., 2000). Cells were subcultured to eliminate contamination by macrophages or other leukocytes and used between passages 3 and 5. Extensive characterization of cell cultures prepared using this protocol previously confirmed >95% purity with cells retaining cytokeskeletal markers of their endometrial stromal origin. For enzyme-linked immunosorbent assay (ELISA) and reverse transcription (RT)–PCR studies, endometrial cells were plated in 12-well plates in Dulbecco’s minimal essential medium F-12 medium, supplied with 10% fetal bovine serum (FBS), 5 μg/ml insulin, 5 μg/ml transferrin and 1% antibiotics-antimycotics. Medium was changed every 48 h. When cells proliferated to confluence, the culture medium was replaced overnight with serum-free medium, then with serum-free and 20% transferrin and 1% antibiotics-antimycotics. Medium was changed every 48 h. When cells proliferated to confluence, the culture medium was replaced overnight with serum-free medium, then with serum-free

Immunocytofluorescence

Endometrial stromal cells were plated on chamber slides (Nalge Nunc International, Naperville, IL, USA). At confluence, cells were incubated overnight with serum-free medium, and then with TNF-α at 1 ng/ml for 6 h. Cells were then fixed in formaldehyde [3.5% v/v in phosphate buffered saline (PBS)] for 15 min at room temperature, rinsed in PBS-0.01% Tween 20, incubated with a goat anti-human MIF antibody [0.66 mg/ml in PBS/0.2% bovine serum albumin (BSA)/0.01% Tween 20] (R & D Systems) for 90 min at room temperature, rinsed in PBS-0.01% Tween 20, incubated with fluorescein isothiocyanate (FITC)-conjugated anti-goat antibody (1/50 dilution in PBS-BSA-Tween) (Jackson ImmunoResearch Laboratories, West Grove, PA, USA), rinsed in PBS-0.01% Tween 20 and mounted in Mowiol containing 10% para-phenylenediamine (Sigma). Cells incubated without the primary antibody or with goat immunoglobulins (Sigma) at the same concentration as the primary antibody were included as negative controls. Slides were observed under a microscope equipped for fluorescence (Leica mikroskopie und systeme GmbH, Model DMRB; Postfach, Wetzlar, Germany) and photographed.

ELISA

MIF ELISA was performed using a mouse monoclonal anti-human-MIF antibody (R & D Systems) as a capture antibody, a rabbit polyclonal anti-human-MIF antibody for detection with an alkaline phosphatase-conjugated goat anti-rabbit antibody and para-nitrophenyl phosphate (Sigma) as substrate (Calandra et al., 1995). The optical density was measured at 405 nm and MIF concentrations were extrapolated from a standard curve using recombinant human MIF. The sensitivity limit of the assay was 300 pg/ml, with intra- and inter-assay coefficients of variation <4%.

RT-PCR

Briefly, following appropriate treatment with TNF-α, cells were washed with ice-cold PBS and extraction of total RNA was performed with Trizol reagent according to the manufacturer’s instructions (Invitrogen). RNA was reverse transcribed in the presence of random primers, and the resulting cDNA was amplified with oligonucleotide primers specific to human MIF (amplimer size 255 bp) and to human glyceraldehyde phosphate dehydrogenase (GAPDH) used as internal control (amplimer size 240 bp). The PCR reaction products were then separated on 1.2% agarose gel by electrophoresis for qualitative analysis of mRNA expression as described previously (Kats et al., 2002b).

Quantitative real-time PCR was performed as described previously (Chung et al., 2002). Each standard PCR reaction contains 2 ml of RT product, 0.5 ml of each primer (final concentration, 5 pmol/l), 12.5 ml SYBR Green PCR Master Mix consisting of Taq DNA polymerase reaction buffer, dNTP mix, SYBR green I, MgCl₂ and Taq DNA polymerase. Following a 10 min denaturation at 95°C, the reactions were cycled 40 times with a 15 s denaturation at 95°C and a 60 s annealing at 59 or 60°C for MIF and GAPDH, respectively. MIF primers (forward, 5’-GGCGCCGGACAGGTCCTACA-3’; reverse, 5’-TTTGCAAGAAGTTGACT-3’; amplimer size 125 bp) (Boeuf et al., 2005), and GAPDH primers (forward, 5’-CAGGCTGCTTAACTCTG-3’; reverse, 5’-TGGTGGAAATCATATT-GGAACA-3’; amplimer size 102 bp) designed with Primer Express™, version 2.0 (Applied Biosystems), span intron-exon boundaries to avoid amplification of genomic DNA, and were selected so as to have compatible Tₘ values (59–61°C). Quantitation of MIF mRNA was performed using a relative quantitation method. For each experimental sample, MIF mRNA levels were normalized to GAPDH mRNA levels. After each run, melting curve analysis (55–95°C) was performed to verify the specificity of the PCR. All samples were tested in

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Western blotting
Briefly, 80 μl lysis buffer (50 mmol/l Tris–HCl, 125 mmol/l NaCl, 0.1% Nonidet-P40, 5 mmol/l ethylenediaminetetraacetic acid, 50 mmol/l NaF, 0.1% phenylmethylsulfonylfluoride and protease inhibitors) was used to extract cytoproteins after collecting cells with cold PBS supplied with phosphorylation inhibitors. Proteins (20 μg) were electrophoretically separated using 10% SDS–PAGE, then transferred onto polyvinylidene difluoride membranes (Millipore Corp., Bedford, MA, USA) overnight at 4°C. After incubation with anti-human p-IκB (Active Motif, Carlsbad, CA, USA) in 0.1% Tween 20–PBS for 1.5 h at room temperature, membranes were incubated with horseradish peroxidase (HRP)-labelled anti-mouse IgGs for 1 h, and a chemiluminescence kit was used for detection following the manufacturer’s instructions (Roche, IN, USA). Membranes were stripped and reblotted with a monoclonal antibody specific to tubulin (Sigma) (1:50 000 dilution in Tween 20–PBS) used as an internal control for protein loading and transfer.

Extraction of nuclear proteins and EMSA
Nuclear proteins were extracted using a nuclear extract kit according to the manufacturer’s instructions (Active Motif) and protein concentration was determined using the Bio-Rad DC Protein assay kit (Bio-Rad Laboratories Ltd, Mississauga, ON, Canada). Nuclear extracts were stored at –80°C until use.

DNA binding of NF-κB was examined using the consensus oligonucleotide of NF-κB (5'AGT TGA GGG GAC TTT CCC AGG C-3'). The oligonucleotide was end-labelled with [γ-32P]ATP (Amersham Pharmacia Biotech) using T4 polynucleotide kinase (Promega, Madison, WI, USA). The labelled probe was purified using a Probe-Quant G-50 micro-column (Amersham Biosciences, Baie d’Urfe, QC, Canada) (3000 r.p.m., 1 min) and recovered in Tris–EDTA buffer pH 8.0. Binding reactions included 5 μg of nuclear proteins in incubation buffer containing 50 mmol/l Tris–HCl pH 7.5, 250 mmol/l NaCl, 2.5 mmol/l dithiothreitol, 2.5 mmol/l EDTA, 5 mmol/l MgCl2, 0.25 mg/ml poly(dl–dC)poly(dl–dC) and 20% glycerol. After 10 min, the labelled oligonucleotide (4 × 105 c.p.m.) was added and the mixture was incubated for 20 min at room temperature in a final volume of 10 μl. When indicated, controls for specific or non-specific competitions were performed using unlabelled NF-κB which was added 20 min before the incubation with the labelled oligonucleotide. Immediately after binding, the nucleoprotein–oligonucleotide complexes were separated from unbound oligonucleotide by electrophoresis on a non-denaturing 4% acrylamide gel at 100 V for 4 h using 0.5 mol/l Tris–borate–EDTA (TBE) buffer. The gel was then dried and exposed overnight at –80°C to ×-ray films (BioMax; Eastman Kodak, Rochester, NY, USA).

Transfections and luciferase assays
Transfections were performed in triplicate in 24-well plates using Plus and LipofectAMINE Reagents (Invitrogen) as described by the manufacturer. Cells were transiently co-transfected with 0.5 μg of human MIF promoter construct in the pGL3 luciferase reporter vector (pGL3-MIF) and 1 μg of human IκB dominant-negative cDNA (S32A/S36A) in the pUSE vector (pUSE-IκB) (Upstate, Charlottesville, VA, USA), or with the appropriate control vectors pGL3 and pUSE. Cells were then washed with PBS and incubated in the culture medium containing 10% FBS overnight before they were stimulated with 1 ng/ml TNF-α for 24 h. Cell extracts were assayed for firefly and renilla luciferase activities using the dual luciferase reporter assay system (Promega) as instructed by the manufacturer.

Statistical analysis
Data were expressed as mean ± SEM. An unpaired t-test was used for comparing two means, and one-way ANOVA followed by the Dunnett’s test was performed for multiple comparisons using GraphPad Software, Prism 3.0 (GraphPad Software, San Diego, CA, USA). Differences were considered as statistically significant whenever a P-value <0.05 occurred.

Results
Analysis of MIF expression in endometrial stromal cells in response to TNF-α was first examined by immunocytofluorescence using anti-MIF antibody. Only a weak staining was observed in cells incubated with the culture medium alone without stimulus (Figure 1A). However, cell incubation with TNF-α (1 ng/ml) for 24 h markedly increased MIF immunostaining (Figure 1B). Staining was virtually absent in cells incubated with goat IgGs instead of anti-MIF antibody or with anti-MIF antibody pre-absorbed with an excess rhMIF (2 μg/ml) (data not shown).

MIF secretion by endometrial stromal cells in response to TNF-α was then quantified by ELISA. As shown in Figure 2, TNF-α induced a dose- and time-dependent increase in MIF concentration in the culture medium. There was no significant increase in MIF secretion in response to TNF-α after 2 h of stimulation. Treatment with 0.1 ng/ml resulted in a progressive increase in MIF secretion, although with no statistical significance. MIF secretion was statistically significant after 6, 12 and 24 h of stimulation with 1 and 10 ng/ml TNF-α (P < 0.05), and appeared to reach a plateau after 6 h of treatment, whereas higher TNF-α concentration (100 ng/ml) induced a statistically significant increase in MIF secretion after 24 h of stimulation (P < 0.05).

The effects of TNF-α on MIF gene expression in endometrial stromal cells were further analysed by RT–PCR. As shown in Figure 3, endometrial stromal cells express detectable levels of MIF mRNA in culture without stimuli. However, adding TNF-α (0–100 ng/ml) to the culture medium for 24 h considerably increased MIF mRNA levels in a dose-dependent manner. Quantitative analysis of MIF mRNA expression using real-time PCR confirmed these data and further showed a significant increase in MIF mRNA levels in endometrial stromal cells in response to 1 (P < 0.01) and 10 (P < 0.05) ng/ml TNF-α.

Figure 1. TNF-α increased MIF expression as detected by immunocytofluorescence. Human endometrial stromal cell cultured to confluence in chamber were incubated for 24 h in culture medium without stimuli (A) or with 1 ng/ml TNF-α (B). Cells were fixed and MIF protein was visualized with goat anti-hMIF and FITC-anti-goat IgGs. Scale bar = 30 μm.
curcumin (culture medium without stimuli), as analysed by the Dunnett’s test. Cells were treated with TNF-α (0, 0.1, 1, 10 and 100 ng/ml) for 24 h. Total RNA was extracted and reverse transcribed, then MIF and GAPDH (internal control) cDNAs were amplified by PCR as described in Material and methods. Analysis of amplified cDNAs by electrophoresis and ethidium bromide staining; (A) Analysis of amplified cDNAs by electrophoresis and ethidium bromide staining; (B) quantitative real-time PCR analysis. MIF mRNA levels were expressed as MIF to GAPDH mRNA ratio. The bars represent means ± SEM; *P < 0.05 versus control (culture medium without stimuli for each incubation time), as analysed by the Dunnett’s test.

Figure 2. TNF-α induced a dose- and time-dependent increase in MIF protein secretion in the culture medium as analysed by ELISA. The bars represent means ± SEM; *P < 0.05 versus control (culture medium without stimuli), as analysed by the unpaired t-test.

Figure 3. TNF-α increased MIF mRNA steady-state levels in cultured human endometrial stromal cells. Cells were treated with TNF-α (0, 0.1, 1, 10 and 100 ng/ml) for 24 h. Total RNA was extracted and reverse transcribed, then MIF and GAPDH (internal control) cDNAs were amplified by PCR as described in Material and methods. (A) Analysis of amplified cDNAs by electrophoresis and ethidium bromide staining; (B) quantitative real-time PCR analysis. MIF mRNA levels were expressed as MIF to GAPDH mRNA ratio. The bars represent means ± SEM; *P < 0.05, **P < 0.01 versus control (culture medium without stimuli), as analysed by the Dunnett’s test.

Figure 4. Curcumin suppressed TNF-α-induced increase in MIF expression in endometrial stromal cells. Confluent cultures of endometrial stromal cells were preincubated for 1 h with or without curcumin (10 nmol/l); TNF-α (1 ng/ml) was then added and the cells were incubated for further 24 h. (A) MIF concentration in the culture supernatant as measured by ELISA; (B) MIF mRNA levels as evaluated by real-time PCR. The bars represent means ± SEM. *Significant stimulation of MIF secretion and mRNA synthesis by TNF-α as analysed by the unpaired t-test (P < 0.05); †P < 0.05 and ††P < 0.01, significant inhibition of TNF-α-induced MIF secretion and mRNA synthesis in the presence of curcumin, as analysed by the unpaired t-test.

Transcription factor NF-κB plays a major role in the regulation of the expression of multiple genes that control the immune system, growth and inflammation (Makarov et al., 1997). NF-κB appeared to play an important role in mediating TNF-α effects in a variety of cell types (Makarov et al., 1997). However, it is still unknown whether NF-κB is involved in MIF gene transcription. Our data showed that curcumin (10⁻⁸ mol/l), an NF-κB inhibitor (Xu et al., 1997; Bharti et al., 2003), significantly decreased the ability of TNF-α to induce MIF protein secretion (P < 0.05) and mRNA synthesis (P < 0.01) (Figure 4).
were exposed to medium without stimuli, 1 ng/ml TNF-α-lysed by electrophoretic mobility shift assay (EMSA). Confluent cell cultures were exposed for 6 h to medium alone, medium with TNF-α or medium with TNF-α (1 ng/ml) following 1 h preincubation with curcumin (10⁻⁸ mol/l). Nuclear protein extracts (5 μg) were incubated with radiolabelled NF-κB oligonucleotide. The resulting complexes were separated on 5% non-denaturing polyacrylamide gel. To test for specificity of NF-κB binding, supershift analysis with antibody against the p50 subunit of NF-κB (NF-κB-Ab) and competition experiments with unlabelled oligonucleotide were conducted.

These results indicate, for the first time, that TNF-α stimulates MIF gene expression and protein synthesis in human endometrial stromal cells and that this effect is mediated by activation of NF-κB. In quiescent cells, NF-κB is bound to IκB in the cytoplasm. Upon stimulation by various agents such as TNF-α, IκB undergoes phosphorylation to form p-IκB, which subsequently undergoes ubiquitylation and degradation. This process allows NF-κB to enter the nucleus, where it activates cytokines, adhesion molecules, cyclooxygenase and other target genes (Makarov et al., 1997). Several compounds are now used as NF-κB inhibitors with different levels of effects. Our data showed that curcumin depressed the ability of TNF-α to increase MIF secretion and inhibited pIκB phosphorylation and NF-κB translocation into the nucleus. Furthermore, co-transfection of endometrial cells with MIF promoter and IκB dominant-negative vectors resulted in a significant inhibition of the TNF-α-induced MIF promoter activity (Figure 7).

Discussion

These results indicate, for the first time, that TNF-α up-regulates MIF gene expression and protein synthesis in human endometrial stromal cells and that this effect is mediated by activation of NF-κB. In quiescent cells, NF-κB is bound to IκB in the cytoplasm. Upon stimulation by various agents such as TNF-α, IκB undergoes phosphorylation to form p-IκB, which subsequently undergoes ubiquitylation and degradation. This process allows NF-κB to enter the nucleus, where it activates cytokines, adhesion molecules, cyclooxygenase and other target genes (Makarov et al., 1997). Several compounds are now used as NF-κB inhibitors with different levels of effects. Our data showed that curcumin depressed the ability of TNF-α to increase MIF secretion and inhibited pIκB phosphorylation and NF-κB translocation into the nucleus. Furthermore, co-transfection of endometrial cells with MIF promoter and IκB dominant-negative vectors resulted in a significant inhibition of the TNF-α-induced MIF promoter activity (Figure 7).
Chegini et al., 1999; von Wolff et al., 1999), thus implicating TNF-α in reproduction.

Several studies have shown the menstrual cycle-dependent production of TNF-α in the human endometrium, its expression in different cell types and the presence of TNF receptors (Hunt et al., 1992; Philippeaux and Piquet, 1993; Tabibzadeh et al., 1995; Chegini et al., 1999; von Wolff et al., 1999). Despite discrepancies in the cyclic expression patterns, these studies suggest a local role for TNF-α in a variety of endometrial functions. This is not surprising, since the human endometrium represents one of the most dynamic tissues during the reproductive age, and cytokines appear to have a crucial role in the regulation of the cyclic events of cell proliferation, remodelling, angiogenesis, apoptosis/necrosis, bleeding and menstrual shedding occurring in this tissue (Tabibzadeh, 1996; 1998; Lebovic et al., 2000; von Wolff et al., 2000; Bigonnesse et al., 2001; Boucher et al., 2001; Critchley et al., 2001). Thus, our study showing an up-regulation of MIF expression by TNF-α in human endometrial cells identifies one of the potential mechanisms underlying MIF expression in the human endometrium, and points to a plausible interaction between these two multifunctional cytokines the local expression and biological properties of which are of particular relevance for the physiology of a tissue like the human endometrium.

MIF was first reported in 1932 and was described as a cytokine produced by T lymphocytes that inhibited the random migration of microphages during delayed hypersensitivity (David, 1966; Bloom and Bennett, 1970). It is now well documented that MIF is a major multifunctional proinflammatory cytokine that activates a number of immunocompetent cells (Metz and Bucala, 1997; Calandra and Roger, 2003; Nishihira et al., 2003) and overrides the anti-inflammatory effects of glucocorticoids (Calandra and Bucala, 1997; Kitaichi et al., 2000). New evidence, however, showed a wider spectrum of action for MIF and an involvement in angiogenesis and tissue remodelling (Metz and Bucala, 1997; Nishihira, 1998; Calandra and Roger, 2003; Nishihira et al., 2003). For instance, MIF was shown to promote directly endothelial cell proliferation in vitro (Chesney et al., 1999; Ogawa et al., 2000; Yang et al., 2000), to stimulate angiogenesis in vivo (Chesney et al., 1999; Nishihira et al., 2003) and to induce the synthesis and the secretion of matrix metalloproteinases in different cell types (Meyer-Siegler, 2000; Onodera et al., 2000), including endometrial cells according to our recent data (A.Akoum and M.J.Therriault, unpublished data). Accordingly, it is quite possible that local induction and production of MIF may contribute to active tissue remodelling and angiogenesis that take place in the endometrial tissue and are required for the tissue’s growth and regeneration, as well as to the inflammatory-like process of leukocyte invasion and menstrual breakdown occurring at the end of the cycle in the absence of implantation (Critchley et al., 2001).

The findings of the present study may have a particular relevance for the understanding of endometriosis, a common gynecological disorder where an endometrial-like tissue creates lesions outside the uterus (Sampson, 1927; Taylor, 2003). Endometriosis is recognized as an inflammatory-like disorder; a chronic immunoinflammatory process has often been described in the peritoneal cavity of endometriosis patients (Halme et al., 1987; Taylor, 2003; Wu and Ho, 2003). Peritoneal fluid from patients with endometriosis was shown to contain high levels of leukocytes, particularly macrophages, and inflammatory cytokines. These cytokines could then contribute to the development of peritoneal endometriosis by promoting endometrial cell adhesion, invasion and proliferation (Hammond et al., 1993; Zhang et al., 1993; Barcz et al., 2000; Wu and Ho, 2003).

Several studies have shown a significant role of TNF-α in endometriosis pathophysiology. Elevated concentrations of TNF-α were found in the peritoneal fluid of women with endometriosis (Rana et al., 1996; Barcz et al., 2000; Iwabe et al., 2000; Bedaivy et al., 2002; Bullimore, 2003; Darai et al., 2003). TNF-α appeared to stimulate metalloproteinase expression (Sillell et al., 2001), to favour cell adhesion to mesothelial cells (Zhang et al., 1993) and to play a considerable role in endometriosis-associated immunoinflammatory changes (Sakamoto et al., 2003). Interestingly, TNF-α appeared to enhance eutopic and ectopic endometrial cell growth (Iwabe et al., 2000; Braun et al., 2002; Sakamoto et al., 2003), but failed to do so in endometrial cells of controls without endometriosis (Braun et al., 2002). This is suggestive of an endometriosis-related intrinsic difference in endometrial cell responsiveness to TNF-α, and in keeping with our previous data showing an increased secretion of monocyte chemotactic protein-1 (MCP-1) in endometrial epithelial cells of women with endometriosis exposed to TNF-α in vitro (Akoum et al., 1995). The effect of TNF-α on MIF secretion by eutopic as well as ectopic endometrial cells of women with endometriosis remains to be elucidated, and it is still to be determined whether these cells had a modified responsiveness to TNF-α with regard to MIF secretion. Nevertheless, our data showing a marked stimulatory effect of TNF-α on endometrial stromal cell secretion of MIF, suggest that peritoneal fluid TNF-α may interact with retrograde endometrial tissue and activate NF-κB, thereby stimulating MIF secretion and exacerbating inflammation. This is all the more plausible since our previous studies showed a marked increase in MIF expression in ectopic endometrial tissue and the peritoneal fluid of endometriosis patients and a relationship with the disease stage (Kats et al., 2002a; b).

In conclusion, our study provides for the first time evidence that TNF-α increases MIF expression in human endometrial stromal cells and that such an effect is mediated by NF-κB. This, owing to the properties of these two major and multifunctional cytokines, may play an important role in the cyclic changes occurring in the human endometrial tissue and potentially contribute to endometriosis pathophysiology.

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