Expression of interleukin-8 and monocyte chemotactic protein-1 in adenomyosis

E.Cagnur Ulukus¹,², Murat Ulukus³,⁴, Yasemin Seval³,⁵, Wenxin Zheng¹ and Aydin Arici³,⁶

Yale University School of Medicine, Departments of ¹Pathology and ³Obstetrics and Gynecology, New Haven, CT, USA, ²Dokuz Eulyul University School of Medicine, Department of Pathology, Inciralti, Izmir, ⁴Ege University School of Medicine, Department of Obstetrics & Gynecology, Bornova, Izmir, and ⁵Akdeniz University School of Medicine, Department of Histology and Embryology, Antalya, Turkey

⁶To whom correspondence should be addressed at: Section of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT 06520-8063, USA. Tel: +1-203-785-3581; Fax: +1-203-785-7134; E-mail: aydin.arici@yale.edu

BACKGROUND: To clarify the inflammatory nature of adenomyosis, we aimed to investigate the expression of interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) by immunohistochemistry to determine their putative role in pathophysiology of adenomyosis. METHODS: Adenomyosis samples, with their eutopic endometrium, were collected from 30 women undergoing hysterectomy. Endometrium from 27 women without adenomyosis were also collected as a control group. Samples were grouped according to the menstrual cycle phase and examined by immunohistochemistry for IL-8 and MCP-1. RESULTS: In normal endometrium, secretory phase samples expressed higher levels of epithelial IL-8 than in proliferative phase samples (P = 0.01), and we observed a trend for an increased epithelial MCP-1 expression in the secretory phase samples compared with the proliferative phase samples (P = 0.07). Endometrial samples of women with adenomyosis did not show the same cyclic variation. In the secretory phase, eutopic endometrium of women with adenomyosis expressed lower levels of epithelial IL-8 and MCP-1 compared with normal endometrium (P < 0.05). The expression of epithelial IL-8 and MCP-1 was higher in the adenomyosis foci than the eutopic endometrium (P < 0.05). CONCLUSIONS: These findings may indicate that an intrinsic abnormality of inflammatory response may be present in eutopic endometrium of women with adenomyosis, and IL-8 and MCP-1 may contribute to the pathophysiology of adenomyosis.

Key words: adenomyosis/endometriosis/endometrium/interleukin-8/monocyte chemotactic protein-1

Introduction

Adenomyosis is defined as the presence of islands of endometrial glands and stroma within the myometrium, whereas endometriosis is the term employed for the presence of endometrial tissue outside the uterus. These two disorders are usually regarded as closely related, but their microscopic appearance, clinical characteristics and probably their pathogenesis are somewhat different. Endometriosis is one of the leading cause of infertility among reproductive age women (Gianetto-Berrutti and Feyles, 2003). However, adenomyosis is not usually associated with infertility, and furthermore, it is more common in parous women (Parazzini et al., 1997). Endometriosis can be diagnosed by either laparoscopy or laporotomy, but the mainstay of the precise diagnosis and treatment of adenomyosis remains hysterectomy.

Since endometriosis is associated with more morbidity than adenomyosis, and adenomyosis can not be diagnosed until the histopathological examination of the hysterectomy specimen, research has been more focused on the pathogenesis of endometriosis. In addition to genetic and environmental factors, alterations in immune and endocrine systems are believed to play important roles in the pathogenesis of endometriosis (Foster and Agarwal, 2002; Seli and Arici, 2003; Bischoff and Simpson, 2004).

Endometrial cells and leukocytes synthesize and secrete multiple cytokines and chemokines that are involved in many physiological reproductive processes such as menstruation, endometrial remodelling and implantation (Garcia-Velasco and Arici, 1999a). To maintain the healthy status of the endometrium, these small molecules act as autocrine and paracrine messengers between endometrial cells and leukocytes. Interleukin-8 (IL-8), known as an α-chemokine with neutrophil chemotactic/activating and T-cell chemotactic activity both in vivo and in vitro, and is believed to be significant in endometrial physiology and pathology. Increased expression of IL-8 by endometrial epithelial cells in the late secretory phase coincides with the neutrophil accumulation in the endometrium just before the onset of menstruation (Arici et al., 1998a). Relatively high levels of IL-8 are also detected during the early to mid proliferative phase of the menstrual cycle, which indicates
that IL-8 may act as a growth and angiogenic factor for the remodelling of endometrium (Arici et al., 1998a).

Monocyte chemotactic protein-1 (MCP-1) is a β-chemokine that specifically induces chemotaxis and activation of mononuclear phagocytes (Sozen et al., 1998). This chemokine is produced by a number of cells including endothelial cells, fibroblasts, monocytes, lymphocytes, smooth muscle cells and even tumour cells (Sozen et al., 1998; Hampton et al., 2001). During the menstrual cycle, endometrial epithelial and stromal cells also express MCP-1 in a similar pattern to IL-8, with higher mRNA levels in the menstrual endometrium (Jones et al., 1997; Milne et al., 1999).

It has been shown that both IL-8 and MCP-1 levels are increased in the peritoneal fluid of women with endometriosis and the levels correlate with the severity of the disease (Akoum et al., 1996; Gazvani et al., 1998; Iwabe et al., 1998; Garcia-Velasco and Arici, 1999a). It has also been demonstrated that IL-8 significantly stimulates cell proliferation in endometrial stromal cells (Arici et al., 1998b). We have demonstrated previously that the attachment of endometrial cells to extracellular matrix stimulates IL-8 and MCP-1 production by these cells (Garcia-Velasco and Arici, 1999b; c, d). All these findings indicate a possible role for these chemokines in the pathogenesis of endometriosis; however, to date there has been no study about IL-8 and MCP-1 in the pathogenesis of adenomyosis.

Since adenomyosis is closely related to endometriosis, we hypothesize that the alterations in IL-8 and MCP-1 expressions may also be involved in the pathogenesis of adenomyosis. To clarify the inflammatory nature of adenomyosis, we aimed to define the expression pattern of these chemokines by immunohistochemistry in adenomyosis and its eutopic endometrium (endometrium overlying adenomyosis) and to compare the levels of these expressions with endometrium of disease-free women.

Materials and methods

Collection of tissues

All retrospective cases in this study were obtained from the Department of Pathology of the Yale University School of Medicine. All tissues were fixed in 10% buffered formalin and processed routinely for paraffin embedding. Tissue samples of adenomyosis, with their corresponding eutopic endometrium, were collected from 30 women undergoing hysterectomy. Age of women ranged from 30 to 52 years old, with an average age of 43 years. Indications for surgery for these women were hypermenorrhea (n = 15), leiomyomata (n = 13) and benign adenial mass (n = 2). Twenty-seven samples of normal endometrium from hysterectomy specimens performed for non-endometrial pathology such as leiomyomata or benign ovarian cysts were also included as controls. These women had no adenomyosis in the histopathological examination of their hysterectomy specimens. The age of women included in the control group ranged from 31 to 53 years old, with an average age of 42.7 years. No woman had any visible pelvic inflammation or endometriosis at the time of hysterectomy. The day of the menstrual cycle was established from the women’s menstrual history and was confirmed by endometrial dating using the criteria of Noyes et al. (1950). Adenomyosis was confirmed by the presence of endometrial glands and stroma within the outer two-thirds of the uterine wall. All women in both study and control groups were premenopausal and had regular menses (lengths varied from 21 to 35 days). All endometrial samples were grouped according to the menstrual cycle phases: proliferative (days 1–14 of the cycle) and secretory (days 15–28 of the cycle). Among eutopic endometrium of women with adenomyosis, 10 were in the proliferative and 20 were in the secretory phase, whereas in the control group, 9 were in the proliferative and 18 were in the secretory phase.

Written informed consent was obtained from each woman before surgery using consent forms and protocols approved by the Human Investigation Committee of Yale University.

Immunohistochemistry

From each case, 5-mm sections were taken onto positively charged slides. The standard streptavidin–avidin immunoperoxidase method was used for immunostaining. The tissue sections were deparaffinized in xylene and rehydrated in alcohol series and immersed in distilled water. Antigen retrieval was performed by boiling the sections in citrate buffer solution (10 mmol/l, pH 6.0) for 30 min. Endogenous peroxidase activity was blocked by 1% H2O2 in phosphate-buffered saline (PBS) at room temperature for 15 min. Slides were incubated with blocking horse serum (LabVision, Fremont, CA, USA) in a humidified chamber for 10 min at room temperature. Excess serum was drained and sections were incubated for 90 min at room temperature in a humidified chamber with the monoclonal antibodies goat anti-human IL-8 (affinity purified goat IgG; R&D Systems, Minneapolis, MN, USA, dilution 1:50) and goat anti-human MCP-1 (affinity purified goat IgG; R&D Systems; dilution 15:100). Negative control sections were incubated with the equivalent protein concentration of goat IgG. Sections were then rinsed in PBS and biotinylated horse anti-goat antibody (1.5 mg/ml; Vector Laboratories, Burlingame, CA, USA) was added at 1:250 dilution for 45 min at room temperature. The antigen–antibody complex was detected using an avidin–biotin–peroxidase kit (LabVision). Diaminobenzidine (3,3-diaminobenzidine tetrahydrochloride dihydrate; LabVision) was used as the chromogen and the sections were counterstained with haematoxylin and mounted with Permount (Fisher Chemicals, Springfield, NJ, USA) on glass slides.

Immunohistochemical staining for IL-8 and MCP-1 was evaluated in a semiquantitative fashion [i.e. 0 (absent staining) to 3 (most intense staining)]. Epithelial and stromal cells were separately evaluated and scored. Myometrium and vascular cells were also evaluated. For each slide, an HSCORE value was derived by summing the percentages of stained cells at each intensity, multiplied by the score of intensity [HSCORE = \( \sum (\pi (i + 1)) \) where \( i \) is the intensity score and \( \pi \) is the corresponding percentage of the cells (McCarty et al., 1986). For each slide 10 different areas were evaluated under the microscope and the percentage of the cells for each intensity within these areas was determined at different times by two investigators blinded to the type and source of the tissues. The average score of two was used.

Statistical analysis

Epithelial and stromal IL-8 and MCP-1 HSCORE values were normally distributed as tested by Kolmogorov–Smirnov test. Differences between proliferative and secretory phase samples were analysed using Student’s t-test. Differences in epithelial and stromal IL-8 and MCP-1 HSCORE values between normal endometrium, eutopic endometrium and adenomyosis samples were analysed using one-way ANOVA test and post-hoc Bonferroni test for pairwise multiple comparisons.

All statistical analyses were performed using Sigmastat for Windows, version 3.0 (Jandel Scientific Corporation, San Rafael, CA, USA). Data are presented as the mean ± SEM. Differences were considered to be significant at \( P < 0.05 \).
Results

IL-8 expression in normal endometrium

In all samples, cytoplasmic IL-8 immunoreactivity was observed throughout the menstrual cycle in both epithelial and stromal cells. Epithelial staining was more intense than the stromal staining in both proliferative and secretory phase of the cycle. However, cytoplasmic staining was prominent on the apical and basal surfaces of the epithelial cells (Figure 1A and B). We did not observe any differences in the staining patterns between the endometrial surface epithelium and the glandular epithelium. There was a cyclic variation of epithelial IL-8 immunoreactivity throughout the menstrual cycle. The HSCORE of the epithelial cells was higher in the secretory phase than in the proliferative phase ($P = 0.01$). No difference was observed in stromal IL-8 immunoreactivity throughout the menstrual cycle (Table I).

Myometrial staining was strong in 19 (70.3%) and weak in 8 (29.6%) samples. Vascular structures were also positively stained with IL-8 in all samples. However, no variation of IL-8 expression was observed in vascular structures during the menstrual cycle.

IL-8 expression in adenomyosis and in its corresponding eutopic endometrium

We observed IL-8 immunoreactivity in all adenomyosis foci and their corresponding eutopic endometrium. Epithelial cytoplasmic staining was more intense than the stromal cytoplasmic staining in both eutopic endometrium and adenomyosis foci. Staining characteristics were similar to normal endometrial samples. However, in eutopic endometrium of women with adenomyosis, epithelial and stromal stainings were less intense than the normal controls (Figure 1C and D). Unlike the normal controls, in the eutopic endometrium of women with adenomyosis, no differences were found in epithelial and stromal HSCORE values between the proliferative and secretory phase samples. Strong and diffuse cytoplasmic staining of epithelial IL-8 was observed in adenomyosis foci (Figure 1E).

When epithelial and stromal IL-8 immunoreactivities were compared between normal endometrium, eutopic endometrium of women with adenomyosis (both phases of the menstrual cycle combined) and the adenomyosis foci, we observed that adenomyosis foci expressed higher levels of epithelial IL-8 compared with their corresponding eutopic endometrium ($P = 0.003$) (Figure 1F; Table I). However, we did not observe such a difference between adenomyosis and normal endometrial samples. In addition, there was no difference in epithelial IL-8 expression between eutopic endometrium of women with adenomyosis and normal controls. Furthermore, no difference was observed in stromal IL-8 expression between adenomyosis foci and their eutopic endometrium, and normal controls.

On the other hand, when we compared eutopic endometrium of women with adenomyosis and control endometrium according

![Figure 1. Representative micrographs of IL-8 staining in normal endometrium, eutopic endometrium of women with adenomyosis and adenomyosis foci. In both eutopic endometrium and adenomyosis samples, epithelial staining was more prominent than stromal staining. In normal endometrium, epithelial IL-8 immunoreactivity was observed on the apical and basal surface of the glands, and secretory phase samples showed higher staining intensity compared with proliferative phase samples (A, proliferative phase; B, secretory phase). In the eutopic endometrial samples of women with adenomyosis, staining characteristics were similar to normal endometrial samples; however, epithelial staining intensity was not different between menstrual cycle phases. Moreover, in secretory phase samples, epithelial immunoreactivity was lower than the normal endometrial samples (C, proliferative phase; D, secretory phase). Strong and diffuse cytoplasmic expression of epithelial IL-8 immunoreactivity was observed in adenomyosis foci (E). Epithelial cells of adenomyosis foci expressed higher levels of IL-8 compared with their eutopic endometrium (F). No positive staining was observed in negative control tissue for IL-8 (G). Scale bars represent 75 mm (A–D); 150 mm (E and G); and 350 mm (F).]

Table 1. HSCORE of IL-8 immunoreactivity in epithelial cells

<table>
<thead>
<tr>
<th>No. of slides</th>
<th>HSCORE (mean ± SEM)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal endometrium</td>
<td>27</td>
<td>279.8 ± 13.2</td>
</tr>
<tr>
<td>Proliferative phase</td>
<td>9</td>
<td>233.2 ± 24.7</td>
</tr>
<tr>
<td>Secretory phase</td>
<td>18</td>
<td>303.1 ± 12.8</td>
</tr>
<tr>
<td>Eutopic endometrium</td>
<td>30</td>
<td>244.4 ± 13.0</td>
</tr>
<tr>
<td>Proliferative phase</td>
<td>10</td>
<td>232.7 ± 24.1</td>
</tr>
<tr>
<td>Secretory phase</td>
<td>20</td>
<td>250.3 ± 15.6</td>
</tr>
<tr>
<td>Adenomyosis</td>
<td>30</td>
<td>303.4 ± 13.7</td>
</tr>
</tbody>
</table>

$^a$Normal secretory endometrium versus normal proliferative endometrium.
$^b$Normal secretory endometrium versus eutopic secretory endometrium.
$^c$Adenomyosis versus eutopic endometrium.
SEM = standard error of the mean.
IL-8 and MCP-1 expression in adenomyosis

to the epithelial and stromal IL-8 immunoreactivity throughout the menstrual cycle, we observed that epithelial IL-8 HSCORE was lower in eutopic endometrium of women with adenomyosis compared with control endometrium in the secretory phase ($P = 0.01$) (Table I). Comparison of functionalis and basalis endometrium revealed a similar result: in secretory phase samples, both endometrial layers of disease-free women expressed higher levels of IL-8 than that of eutopic endometrium of women with adenomyosis. Data are summarized in Table I.

Myometrial staining was strong in 27 (90%) and weak in 3 (10%) samples. Vascular structures were also positively stained with IL-8 in all samples. Similar to normal endometrial samples, no variation of IL-8 expression was observed in either eutopic endometrium or adenomyosis foci.

No positive staining was observed in negative control tissue for IL-8 (Figure 1G).

**MCP-1 expression in normal endometrium**

Cytoplasmic MCP-1 immunoreactivity was observed throughout the menstrual cycle in all endometrial samples. Stromal cells were stained with a relatively less intensity compared with the epithelial cells. In most samples, epithelial MCP-1 staining was more intense at the apical and basal surface of the cells (Figure 2A and B). We did not observe any differences in the staining patterns between the endometrial surface epithelium and the glandular epithelium. There was a trend for increased epithelial MCP-1 expression in the secretory phase compared with the proliferative phase in normal endometrium ($P = 0.07$) (Table II). No difference was observed in stromal MCP-1 expression throughout the menstrual cycle.

Myometrial MCP-1 staining was strong in 7 (25.9%) and weak in 18 (66.6%) samples. In 2 (7.4%) cases, no myometrial staining was observed. However no variation of MCP-1 expression was observed in vascular structures during the menstrual cycle.

**MCP-1 expression in adenomyosis and in its corresponding eutopic endometrium**

Similar to normal endometrial samples, we observed MCP-1 staining in all adenomyosis foci and their corresponding eutopic endometrium. Stromal cells were stained with less intensity compared with epithelial cells in both eutopic endometrium and adenomyosis. Staining characteristics were similar to normal endometrial samples. Unlike the normal controls, in the eutopic endometrium of women with adenomyosis, epithelial MCP-1 immunoreactivity was higher in the proliferative phase compared with the secretory phase ($P = 0.02$) (Figure 2C and D; Table II). We observed strong and diffuse epithelial MCP-1 immunoreactivity in adenomyosis foci (Figure 2E).

When we compared epithelial and stromal MCP-1 expression between normal endometrium, eutopic endometrium of women with adenomyosis (both phases of the menstrual cycle combined) and adenomyosis foci, we observed that adenomyosis foci expressed higher levels of epithelial MCP-1 compared with their corresponding eutopic endometrium ($P = 0.04$) (Figure 2F; Table II). However, we have not observed such a difference between adenomyosis foci and normal endometrial samples. In addition, there was no difference in epithelial or stromal MCP-1 expressions between eutopic endometrium of women with adenomyosis and normal controls.

On the other hand, when we compared eutopic endometrium of women with adenomyosis and control endometrium according to the epithelial and stromal MCP-1 immunoreactivity throughout the menstrual cycle, we observed that in the secretory...
phase, HSCORE of epithelial cells was lower in eutopic endometrium of women with adenomyosis compared with control endometrium ($P = 0.02$). Comparison of functionalis and basalis endometrium revealed similar results: both endometrial layers of disease-free women expressed higher levels of MCP-1 than that of women with adenomyosis. Data are summarized in Table II.

Myometrial staining was strong in 11 (36.6%) and weak in 18 (63.3%) samples. No staining was observed in one (3.3%) sample. Vascular structures were also positively stained in most of the samples, although the staining intensity was not as strong as IL-8. Similar to normal endometrial samples, no variation of MCP-1 expression was observed in either eutopic endometrium or adenomyosis foci.

No positive staining was observed in negative control tissue for MCP-1 (Figure 2G).

### Discussion

In contrast to endometriosis, being characterized by the presence of ectopic endometrium outside the uterus, adenomyosis is defined as the presence of endometrial glands and stroma within the myometrium. In recent years, many studies have revealed the inflammatory nature of endometriosis. Increased number of immune cells and their secretory products, cytokines and chemokines, have been detected in the peritoneal fluid of women with endometriosis (Harada et al., 2001; Lebovic et al., 2001; Berkkanoglu and Arici, 2003). IL-8, a potent angiogenic and chemotactic factor for neutrophils, is the representative of the α-chemokines, and MCP-1, a potent chemotactic factor for monocytes, is the representative of the other major subfamily of chemokines, β-chemokines (Sozen et al., 1998; Senturk et al., 2001). It has been shown that ectopic endometrial cells are also a source of IL-8 and MCP-1, as well as immune and normal endometrial cells (Arici et al., 1998b; Iwabe et al., 1998; Akoum et al., 2000). It is strongly believed that these secretory products play important roles in the establishment and maintenance of the disease by stimulating the attachment of endometrial cells to extracellular matrix and proliferation of endometriotic cells (Akoum et al., 1996; Garcia-Velasco and Arici, 1999d).

To date, there are no published data about the inflammatory characteristics of adenomyosis concerning IL-8 and MCP-1 expression. Our findings about the expression of IL-8 and MCP-1 in normal endometrium are consistent with previous studies (Jones et al., 1997; Arici et al., 1998a). Epithelial expressions of these two chemokines show cyclic variation and increase during the secretory phase compared with the proliferative phase. This finding suggests that these chemokines may regulate some physiological endometrial functions under the influence of sex steroid hormones. As reported previously, the increase in the expression of both chemokines in the late secretory phase coincides with the accumulation of leukocytes into the endometrium just before the onset of menstruation (Jones et al., 1997; Arici et al., 1998a).

On the other hand, we did not observe such cyclic variation in the expression of the two chemokines in the eutopic endometrium of women with adenomyosis. Furthermore, MCP-1 expression was significantly higher in the proliferative phase compared with the secretory phase. In addition, in eutopic endometrial samples of women with adenomyosis, the staining intensity of both chemokines was significantly lower in the secretory phase compared with normal endometrial samples. This finding suggests that eutopic endometrium might be affected by the presence of adenomyosis. This finding may also indicate that an intrinsic abnormality of inflammatory response may be present in eutopic endometrium of women with adenomyosis. In other words, IL-8 and MCP-1 may have different effects on the eutopic endometrium of women with adenomyosis compared with the normal endometrium. Although immunohistochemistry provides only a semi-quantitative evaluation, which may limit the value of our findings, immunohistochemistry reveals the precise localization of each chemokine in eutopic endometrium and in adenomyotic foci. However, their identification with quantitative techniques such as mRNA evaluation will provide further support to our findings.

Our study revealed that epithelial cells of adenomyosis foci express significantly higher levels of IL-8 and MCP-1 compared with their corresponding eutopic endometrium, although these levels are not higher than that of the normal endometrium. The local cytokine and steroid hormone interactions, even in the same uterus, may play a putative role in this difference. Recently, it has been demonstrated that both eutopic and ectopic endometrium of women with endometriosis and adenomyosis express aromatase and estrone sulphatase, which catalyse the local estrogen production in these areas (Yamamoto et al., 1993). Aromatase expression was shown by immunohistochemistry in the cytoplasm of glandular cells of both eutopic and ectopic endometrium of adenomyosis (Yamamoto et al., 1993). Moreover, estrogen and progesterone receptors have been detected in adenomyotic tissues (Ferenczy, 1998). These findings support the view that adenomyosis is an estrogen-dependent disease. Furthermore, the different levels of estrogen between eutopic and ectopic endometrium of women with adenomyosis may also determine the expression characteristics of IL-8 and MCP-1 in these tissues.

We have shown previously that IL-8 and MCP-1 chemokines are also expressed by myometrial cells. We observed that the MCP-1 mRNA levels in the myometrium of women with leiomyomata was higher than in the myometrium of control women (Sozen et al., 1998). We also showed a strong immunostaining

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**Table II. HSCORE of MCP-1 immunoreactivity in epithelial cells**

<table>
<thead>
<tr>
<th></th>
<th>No. of slides</th>
<th>HSCORE (mean ± SEM)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Normal endometrium</td>
<td>27</td>
<td>198.8 ± 9.2</td>
<td></td>
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<tr>
<td>Proliferative phase</td>
<td>9</td>
<td>175.4 ± 18.2</td>
<td></td>
</tr>
<tr>
<td>Secretory phase</td>
<td>18</td>
<td>210.4 ± 9.7</td>
<td>0.07a</td>
</tr>
<tr>
<td>Eutopic endometrium</td>
<td>30</td>
<td>189.5 ± 2.6.4</td>
<td></td>
</tr>
<tr>
<td>Proliferative phase</td>
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<td>209.6 ± 6.0</td>
<td>0.02b</td>
</tr>
<tr>
<td>Secretory phase</td>
<td>20</td>
<td>179.5 ± 8.3</td>
<td>0.02c</td>
</tr>
<tr>
<td>Adenomyosis</td>
<td>30</td>
<td>211.1 ± 7.7</td>
<td>0.04d</td>
</tr>
</tbody>
</table>

a Normal secretory endometrium versus normal proliferative endometrium.
b Eutopic proliferative endometrium versus eutopic secretory endometrium.
c Normal secretory endometrium versus eutopic secretory endometrium.
d Adenomyosis versus eutopic endometrium.

SEM = standard error of the mean.
for both IL-8 and IL-8 receptor type A in leiomyomatous uterus (Senturk et al., 2001). Since adenomyosis is generally associated with leiomyomata, myometrial IL-8 and MCP-1 expressions may induce chemokine expression in adenomyosis foci. In our series, almost half of the women with adenomyosis also had leiomyomata. This may provide another explanation why higher IL-8 and MCP-1 immunoreactivities in adenomyosis foci compared with eutopic endometrium were found in the present study. We also observed IL-8 expression in the vascular structures of both normal and adenomyotic uteri. As was reported in earlier studies, this chemokine may also be involved in angiogenesis (Senturk et al., 2001).

Recently, Sotnikova et al. (2002) investigated cytokine synthesis by mononuclear cells infiltrating eutopic and ectopic endometrium of women with adenomyosis. They found that in eutopic endometrium of women with adenomyosis, IL-8 levels in the supernatant of mononuclear cells was significantly reduced compared with that of the control samples. In contrast, they observed significantly lower levels of IL-8 content in ectopic endometrial mononuclear cells. The findings of Sotnikova et al. and of the present study suggest that ectopic endometrial cells and immune cells infiltrating ectopic endometrium may express different levels of IL-8. Interactions between immune cells and ectopic endometrium, as well as normal endometrium, may be an explanation for this finding.

In conclusion, to our knowledge this is the first study that investigates the expression of these chemokines in adenomyotic uteri. Our results suggest that IL-8 and MCP-1 are expressed differently in the eutopic endometrium of women with adenomyosis compared with normal endometrium, and epithelial cells of adenomyosis foci expressed significantly higher levels of these chemokines compared with their corresponding homologous endometrium. Although these results indicate that IL-8 and MCP-1 may be involved in the pathogenesis of adenomyosis, their identification alone limits to make further interpretation. However, further studies involving their receptor expression will clarify the inflammatory nature of adenomyosis.

Acknowledgements
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