Growth-Regulated α Expression in the Peritoneal Environment with Endometriosis

ENGIN ORAL, MD, EMRE SELI, MD, MERT O. BAHTIYAR, MD, DAVID L. OLIVE, MD, AND AYDIN ARICI, MD

Objective: **To investigate the presence and modulation of growth-regulated α, a member of the chemokine family with neutrophil chemotactic activity, in the peritoneal fluid of women with or without endometriosis.**

Methods: **Peritoneal fluid samples were obtained at laparoscopy from 63 women with endometriosis and 19 fertile women without endometriosis. Endometrial tissue was obtained from uteri after hysterectomy for reasons other than endometrial disease or from endometrial biopsies of reproductive-age women. Cellular RNA was extracted and northern blots were hybridized with an oligonucleotide probe complementary to a specific sequence of growth-regulated α messenger RNA. Growth-regulated α in peritoneal fluid and culture supernatant was quantified using enzyme-linked immunosorbent assay. Statistical analyses were performed using Kruskal-Wallis and Mann-Whitney tests.**

Results: **The median (range) concentration of growth-regulated α in peritoneal fluid samples from 19 normal fertile women was 27 pg/mL (0–108), from 24 women with moderate endometriosis 34 pg/mL (8–150), and from seven with severe endometriosis was 73 pg/mL (10–221) ($P = .04$, $P = .01$, respectively). In the moderate and severe endometriosis groups, the levels of growth-regulated α were significantly higher in the peritoneal fluid of women with untreated endometriosis (73 pg/mL [10–221]) than in women with medically treated endometriosis (25 pg/mL [8–47]). In mesothelial and endometrial stromal cells in culture, growth-regulated α messenger RNA and protein were detectable constitutively; however, both interleukin-1α and tumor necrosis factor-α induced higher levels of growth-regulated α messenger RNA and protein in a dose- and time-dependent manner.**

Conclusions: **Growth-regulated α levels are elevated in the peritoneal fluid of women with moderate and severe endometriosis. This chemotactic factor, which acts via the interleukin-8 receptor, may play a role in the pathogenesis of endometriosis.** *(Obstet Gynecol 1996;88:1050–6.*

From the Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut.

Endometriosis, one of the most common benign gynecologic conditions, has an estimated prevalence of 10% among reproductive age women.[1] Considerable circumstantial evidence supports the role of retrograde menstruation with subsequent endometrial implantation as an etiologic factor in most cases.[2] Retrograde menstruation is a nearly universal phenomenon among cycling women,[3] but it is not clear why endometrial tissue will implant and grow in the peritoneal cavity in only a subgroup of women.

Immunologic dysfunction has been associated with endometriosis. Peritoneal fluid creates an immunologically dynamic environment linking the reproductive and immune systems. The cellular compartment of the peritoneal fluid is composed mainly of macrophages. In the peritoneal fluid of endometriotic women, an increased number, concentration, and activation of macrophages has been described.[4] Secretory products of macrophages such as interleukin-1 (IL-1),[5] tumor necrosis factor-α (TNF-α),[6] and growth factors[7] are also found in increased quantities in the peritoneal fluid of these women.

One study[8] has shown increased neutrophil chemotactic activity in peritoneal fluid of women with endometriosis. However, the nature and source of this chemotactic factor remains to be determined. Several structurally related peptides capable of inducing neutrophil chemotaxis have been identified.[9] Recently, we identified the presence of interleukin-8 (IL-8), a chemoattractant cytokine for neutrophils, in the peritoneal fluid of women with endometriosis.[10] Another chemotactic factor candidate is growth-regulated α, which is reported to be ten times more potent than IL-8 as a neutrophil chemoattractant.[11] Growth-regulated α gene expression has been demonstrated in fibroblasts, endothelial cells, and activated neutrophils and monocytes.[9,12] Two other subtypes of the growth-regulated family, growth-regulated β and growth-regulated γ,

 0029-7844/96/$15.00
PII S0029-7844(96)00361-4

have been found to share 90% and 86% of the nucleotide sequences with growth-regulated α, respectively.[13]

We postulated that growth-regulated α may act as an important modulator in the pathogenesis of endometriosis by attracting and activating neutrophils. We investigated the presence of growth-regulated α in peritoneal fluid of women with or without endometriosis and the possibility of a correlation between the peritoneal fluid growth-regulated α concentration and the severity of endometriosis. We then assessed peritoneal, mesothelial, and endometrial cells as potential sources of peritoneal fluid growth-regulated α.

Materials and Methods

Tissue Collection

Peritoneal fluid samples were obtained from women undergoing diagnostic laparoscopy at Yale-New Haven Hospital between 1993 and 1995. Indications for laparoscopy included evaluation of infertility, pelvic pain, or elective tubal sterilization. During the study period, all samples obtained from women with endometriosis and samples selected randomly from women undergoing laparoscopic tubal ligation were included. Informed consent was obtained from each woman before surgery using consent forms and protocols approved by the human investigation committee of this university. Menstrual cycle dates were determined from each woman's menstrual history and verified in a subset of women by histologic examination of the endometrium. Women were classified into five groups, according to anatomic findings observed during the procedure: normal fertile women (undergoing laparoscopic tubal ligation, n = 19), and women with minimal (n = 13), mild (n = 19), moderate (n = 24), and severe (n = 7) endometriosis as defined by the revised American Fertility Society classification.[14] All women with appearance of endometriosis at diagnostic laparoscopy underwent biopsies that confirmed the disease. Women undergoing laparoscopic tubal ligation with no gross evidence of disease did not have biopsies. Those with a diagnosis of moderate-to-severe endometriosis (n = 6), in whom surgical procedures were performed as an evaluation of continuing medical treatment for the disease, were also included. This group was receiving GnRH agonist (Lupron Depot, TAP Pharmaceuticals, North Chicago, IL) for at least 6 months before surgery.

After insertion of the laparoscope and ancillary trocars, peritoneal fluid was aspirated immediately to avoid blood contamination. Fluid from the anterior and posterior cul-de-sacs was collected into a sterile syringe and transferred immediately to the laboratory, where it was centrifuged at 600 × g for 10 minutes at 4C to remove cells. Aliquots were frozen at −80C until assayed. Cell pellets were resuspended in Hank's balanced salt solution for mesothelial cell culture.

Endometrial tissue was obtained from uteri after hysterectomy for reasons other than endometrial disease or from endometrial biopsies of reproductive-age women. Endometrial tissue was placed in Hank's balanced salt solution and transported to the laboratory for separation and culture of endometrial stromal cells and glandular epithelium.

Isolation and Culture of Human Mesothelial and Endometrial Cells

Mesothelial cells were isolated and cultured as described previously,[10] plated in culture dishes (10 mm diameter or 24-well plates) as appropriate for the experimental design, and allowed to replicate to confluence before each experiment. Mesothelial cells grew, forming a homogenous population after three passages, and containing less than 1% macrophages. All experiments were performed using cells at the third passage. Experiments commenced 1 to 3 days after confluence was attained. All experiments were conducted using serum-free, epidermal growth factor-free media.

Endometrial stromal and epithelial cells were separated and maintained in monolayer culture as described previously.[15] Stromal cells, after the first passage, were plated in culture dishes (100-mm diameter or 24-well plates) as appropriate for the experimental design and allowed to replicate to confluence. After first passage, endometrial stromal cells contained up to 7% epithelial cells, no endothelial cells, and 0.2% macrophages. Experiments commenced 1–3 days after confluence was attained. Experiments with endometrial glandular cells were conducted after 4–5 days of primary culture. The confluent cells were incubated with serum-free media for 24 hours before initiation of treatment with test agents.

At the end of each experiment, the culture media were collected and frozen at −80C for quantification of growth-regulated α by enzyme-linked immunosorbent assay (ELISA). Cells were used for RNA isolation or total protein measurement.

Preparation of Total RNA and Northern Analysis

Total RNA was extracted from cells in culture using Trizol (Gibco BRL, Grand Island, NY). Total RNA (5 or 10 μg per lane) was size-fractionated by electrophoresis on 1% formaldehyde-agarose gels, transferred electrophoretically to Hybond-N^+ membrane (Amersham, Arlington Heights, IL), and cross-linked to the membrane using ultraviolet light. Prehybridization was conducted

for 5 hours at 65C in buffer comprised of NaCl (0.9 M), Tris-Cl (90 mM, pH 8.3), ethylenediaminetetra-acetic acid [EDTA] (6 mM), 5× Denhardt solution, sodium dodecylsulphate (0.1%), sodium pyrophosphate (0.1% weight per volume), and salmon sperm DNA (0.2 mg/mL). Hybridizations were conducted for 16 hours at 65C in a buffer that contained a growth-regulated α-specific oligonucleotide probe (5′-GGC ATG TTG CAG GTC CCT CA-3′) end-labeled with [γ-^{32}P]ATP; the sequence of this probe corresponds to the portion between bases 714 and 695 of the growth-regulated α gene.[16] Thereafter, the blots were washed with 6× standard saline citrate and sodium dodecylsulphate (0.1% weight per volume) for 15 minutes at room temperature, once with 2× standard saline citrate and sodium dodecylsulphate (0.1% weight per volume) for 15 minutes at room temperature, and once for 20 minutes at 65C. Autoradiography of the membranes was performed at −70C using Kodak X-Omat AR film (Eastman Kodak, Rochester, NY). The amount of RNA in each lane was normalized by analysis of glyceraldehyde-3-phosphate dehydrogenase messenger RNA, using a complementary DNA probe (Clontech Laboratories, Palo Alto, CA) radiolabeled with [α-^{32}P]dCTP by random hexamer priming. The autoradiographic bands were quantified using a laser densitometer (Molecular Dynamics, Sunnyvale, CA). Growth-regulated α in each band was corrected by using the value for the corresponding glyceraldehyde-3-phosphate dehydrogenase messenger RNA. Similar experiments were conducted on three different occasions with cells prepared from different peritoneal fluids.

ELISA

Immunoreactive growth-regulated α in peritoneal fluid samples and culture supernatant from mesothelial and endometrial cells were quantified using an ELISA (R&D Systems, Minneapolis, MN). According to the manufacturer, there is no measurable cross-reactivity with other known cytokines in this assay, and our experience with this assay confirms the absence of cross-reactivity with IL-1α or TNF-α. The limit of detection for growth-regulated α was 5 pg/mL. All of the peritoneal fluid samples were evaluated in a duplicate assay. The assay's use for human peritoneal fluid was validated; recombinant growth-regulated α was diluted in assay buffer, and pooled peritoneal fluid and parallelism were observed between the standard curve of buffer and peritoneal fluid dilutions. Each experiment was done using three replicate wells for each condition, and supernatant from each well was tested in a single ELISA. Each experimental setup was repeated on at least three occasions, using mesothelial and endometrial cells obtained from three different women. The individuals doing the assays were blinded to clinical results. The intra- and interassay coefficients of variation were 4.4 and 8.1%, respectively.

Immunoreactive IL-8 in peritoneal fluid samples was quantified using an ELISA from R&D Systems. According to the manufacturer, there is no measurable cross-reactivity with other known cytokines in this assay. The limit of detection for IL-8 was 3 pg/mL. All of the peritoneal fluid samples were evaluated in a duplicate assay, and the use of IL-8 assay for human peritoneal fluid was validated: recombinant IL-8 was diluted in assay buffer, and pooled peritoneal fluid and parallelism were observed between the standard curve of buffer and peritoneal fluid dilutions. The intraassay and interassay coefficients of variation were 3.3 and 10.2%, respectively.

Statistical Analyses

Because the growth-regulated α levels in the peritoneal fluid were not normally distributed, they were analyzed with nonparametric analysis of variance by ranks (Kruskal-Wallis test) and within group differences by post hoc analysis using the Mann-Whitney test. Enzyme-linked immunosorbent assay results were analyzed by analysis of variance, and within group differences by post hoc analysis Bonferroni correction for multiple correction. Correlation analysis by ranks was calculated with the Spearman correlation coefficient. $P < .05$ was considered statistically significant. The statistical analysis was performed with the Statistical Package for Social Sciences, Version 6.0 for Windows (SPSS Inc., Chicago, IL).

Results

Immunoreactive Growth-Regulated α in Peritoneal Fluid

Table 1 summarizes the clinical profile of the study participants. In women with endometriosis, we observed a stage-dependent elevation in the peritoneal fluid growth-regulated α concentration. In peritoneal fluid samples from women with minimal endometriosis ($n = 13$), the median growth-regulated α level was 25 pg/mL (range, 3–70); with mild endometriosis ($n = 19$) it was 14 pg/mL (0–107); with moderate endometriosis ($n = 24$) the level was 34 pg/mL (8–150); and with severe endometriosis ($n = 7$) it was 73 pg/mL (10–221). In women without endometriosis ($n = 19$), the median level was 27 pg/mL (0–108). A statistically significant difference among the five groups was observed ($P = .01$), with post hoc analysis revealing a difference be-

Table 1. Clinical Characteristics of Subjects

	Normal (n = 19)	Endometriosis (n = 63)			
		Minimal (n = 13)	Mild (n = 19)	Moderate (n = 24)	Severe (n = 7)
Age*	30.3 ± 5.9	33.8 ± 5.5	32.1 ± 5.1	32.0 ± 5.3	37.1 ± 7.9
Parity*	2.4 ± 0.7	0.7 ± 0.2	0.9 ± 0.3	0.6 ± 0.2	0.4 ± 0.1
Infertility†	0	6 (46)	11 (57.9)	11 (46)	3 (42.9)
Chronic pelvic pain†	0	7 (54)	7 (36.8)	13 (54)	3 (42.9)

* Values are means ± standard deviation.
† Values in parentheses are percentages.

tween normal women (controls) and women with moderate endometriosis (P = .04), and controls and women with severe endometriosis (P = .01). Rank correlation analysis demonstrated a statistically significant relationship between growth-regulated α level and severity of disease (r = .36, P = .003). Within the moderate and severe endometriosis groups (n = 31), the levels of growth-regulated α in the peritoneal fluid of women with untreated endometriosis were higher (73 pg/mL [10–221]) than in women who had undergone medical treatment with GnRH agonist (25 pg/mL [8–47]), (P = .04).

We then tested whether growth-regulated α levels were menstrual-cycle dependent. Accurate menstrual dating at the time of peritoneal fluid collection was available in 53 of the 82 study subjects. In women without endometriosis, the median concentration of growth-regulated α in the proliferative phase was 30 pg/mL (range, 23–43) (n = 7); in the secretory phase, it was 0 pg/mL (range, 0–70) (n = 5). In women with endometriosis, these values were 25 pg/mL (0–221) (n = 27) and 31 pg/mL (3–107) (n = 14), respectively. No significant correlation between cycle phase and growth-regulated α concentration was observed in the endometriosis or the control group. Because growth-regulated α and IL-8 share a common receptor, we investigated for correlation of the levels in the peritoneal fluid. In the fertile women without endometriosis, there was no correlation between levels of these cytokines in peritoneal fluid. However, in the endometriosis group (n = 63), there was a correlation between peritoneal fluid growth-regulated α concentrations and IL-8 concentrations (r = .59, P < .001).

Growth-Regulated α Production by Mesothelial Cells

Confluent mesothelial cells were incubated with human recombinant IL-1α (10 U/mL) or TNF-α (10 ng/mL) for 6 hours. Growth-regulated α messenger RNA was detectable by Northern analysis in nontreated cells. Both IL-1α and TNF-α induced higher levels of growth-regulated α messenger RNA (Figure 1). An increase in the growth-regulated α messenger RNA levels in mesothelial cells was seen with increasing concentration of IL-1α (0.1–100 U/mL) and TNF-α (0.1–100 ng/mL) (data not shown). In mesothelial cells treated with IL-1α (10 U/mL), the level of growth-regulated α messenger RNA increased after 2 to 9 hours of IL-1α treatment and then decreased (data not shown).

Untreated mesothelial cells in culture produced and secreted growth-regulated α protein, as quantified by ELISA (Figure 2). Treatment with IL-1α (10 U/mL) or TNF-α (10 ng/mL) caused increases in the accumulation of immunoreactive growth-regulated α in the media; the difference was statistically significant by 8 hours of treatment (P = .001). After 24 hours of IL-1α and TNF-α treatment, the longest duration of the experiments, maximal accumulation of growth-regulated α in the media was observed (P < .001). Similarly, a lower increase in the production of growth-regulated α was observed when cells were treated at a lower concentra-

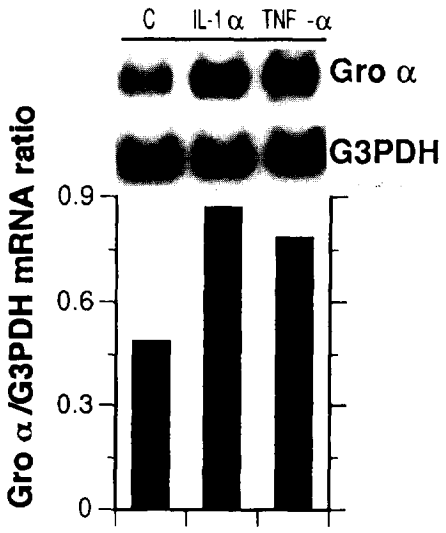

Figure 1. Northern analysis of growth-regulated α (Gro α) messenger RNA in mesothelial cells treated with interleukin-1 alpha (IL-1α) and tumor necrosis factor-alpha (TNF-α). Confluent mesothelial cells in culture were incubated for 6 h with IL-1α (10 U/mL) and TNF-α (10 ng/mL). Total RNA (10 μg per lane) was evaluated. C = control; G3PDH = glyceraldehyde-3-phosphate dehydrogenase.

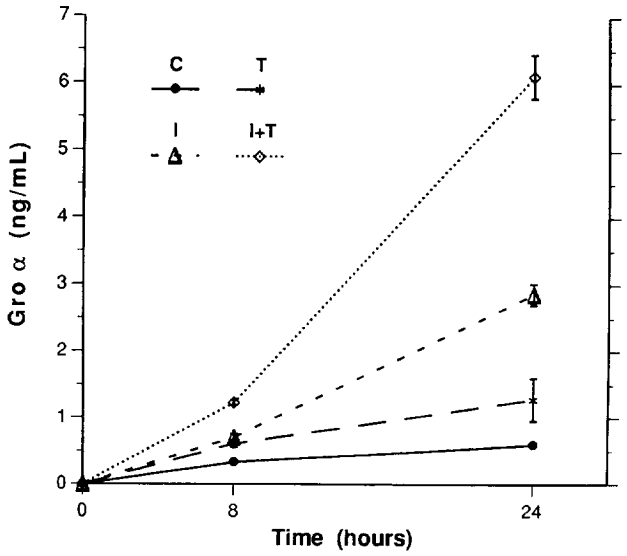

Figure 2. Stimulation of immunoreactive growth-regulated α (Gro α) production by mesothelial cells in culture by interleukin-1 alpha (IL-1α) and tumor necrosis factor-alpha (TNF-α). Confluent mesothelial cells were treated with serum-free medium alone (control) or with medium containing IL-1α (10 U/mL) or TNF-α (10 ng/mL) or both for 8 and 24 h. The culture media were collected, and growth-regulated α was quantified by enzyme-linked immunosorbent assay. Data are mean ± standard error of the mean for four replicates. C = control; I = IL-1α; T = TNF-α.

tion of IL-1α (1 U/mL) or TNF-α (1 ng/mL) (data not shown).

Growth-Regulated α Production by Endometrial Cells

Fetal bovine serum (0.5–10% volume per volume) caused a concentration-dependent increase in the levels of growth-regulated α in endometrial stromal cells in culture. The serum also induced an increase in the levels of growth-regulated α messenger RNA in IL-1α-treated (10 U/mL) cells. Consequently, we removed serum from the culture medium 24 hours before the initiation of all experiments.

The level of growth-regulated α messenger RNA was low but detectable in stromal cells. The growth-regulated α messenger RNA level increased markedly in response to IL-1α and TNF-α treatment. This increase was evident by 30 minutes, and high levels of growth-regulated α messenger RNA were present by 6 hours following both treatments; thereafter, levels began to decrease (Figure 3). The increase in the level of growth-regulated α messenger RNA in stromal cells was dependent on the concentration of IL-1α (0.01–100 U/mL) or TNF-α (0.01–100 ng/mL) (data not shown).

Untreated endometrial stromal cells in culture produced and secreted growth-regulated α protein quantified by ELISA (Figure 4). Treatment of endometrial stromal cells with IL-1α (10 U/mL) or TNF-α (10 ng/mL) resulted in increases in the accumulation of immunoreactive growth-regulated α; these differences were significant by 8 hours (P = .001 for IL-1α treated versus control). After 24 hours of IL-1α and TNF-α treatment, the longest duration of the experiments, maximal accumulation of growth-regulated α in the media was observed (P = .001 for IL-1α treated versus control, P = .01 TNF-α treated versus control).

Growth-regulated α messenger RNA was not detected by Northern analysis of total RNA isolated from endometrial glandular cells after 4 days in monolayer culture.

Discussion

The evidence suggesting that peritoneal fluid in women with endometriosis is pro-inflammatory is compelling. Olive et al[17] have demonstrated that endometrial stromal cell growth can be optimized in an environment of estrogen and growth factors, particularly those growth factors secreted by the peritoneal macrophages; this is consistent with the finding of increased macrophage number and activation level in the peritoneal cavity of women with endometriosis.[4] Given this scenario, we asked whether the macrophage abnormality in women

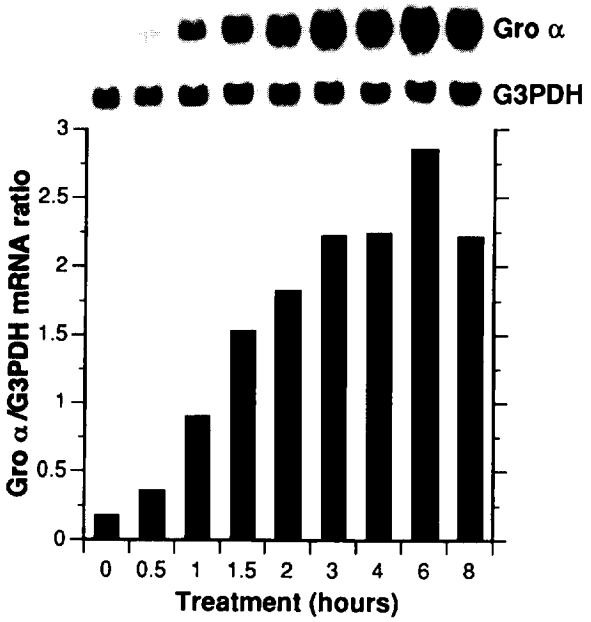

Figure 3. Time course of induction of growth-regulated α (Gro α) messenger RNA in endometrial stromal cells by interleukin-1 alpha (IL-1α). Confluent endometrial stromal cells in culture were incubated for 8 h in serum-free culture medium with IL-1α (10 U/mL). Total RNA (10 μg per lane) was evaluated. G3PDH = glyceraldehyde-3-phosphate dehydrogenase.

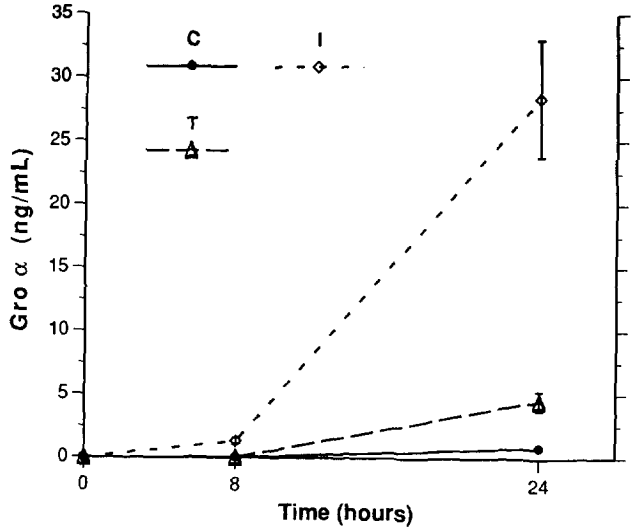

Figure 4. Stimulation of immunoreactive growth-regulated α (Gro α) production by endometrial stromal cells in culture by interleukin-1 alpha (IL-1α) and tumor necrosis factor-alpha (TNF-α). Confluent mesothelial cells were treated with serum-free medium alone (control) or with medium containing IL-1α (10 U/mL) or TNF-α (10 ng/mL) for 8 and 24 h. The culture media were collected, and growth-regulated α was quantified by enzyme-linked immunosorbent assay. Data are mean ± standard error of mean for four replicates. C = control; I = IL-1α; T = TNF-α.

with endometriosis might represent the primary pathogenetic factor or whether a pre-existing condition promotes leukocyte migration and activation.

One candidate for the latter possibility is the chemokine. The chemokines are a recently described family of inflammatory proteins; they are related on the basis of their primary structure, notably the conservation of a four-cysteine residue motif.[11] Two chemokine families exist—the C-C family, in which the first two cysteine residues are adjacent to one another; and the C-X-C family, in which there is an intervening amino acid between the first two cysteine residues. The C-X-C family, which includes IL-8, growth-regulated α, neutrophil-activating protein-2, platelet factor-4, and γ-interferon-inducible protein-10, is predominantly chemotactic for neutrophils. We have shown recently that one member of this family, IL-8, is elevated in the peritoneal fluid of women with endometriosis.[10]

Growth-regulated α, a chemokine of 73 amino-acid residues, was described originally as a mitogen for human melanoma cells (melanoma growth stimulatory activity), and subsequently it was found to have pro-inflammatory activity, leading to neutrophil recruitment and activation.[18,19] Interleukin-8 and growth-regulated α exert their biologic influences by binding to specific cell receptors. The type A IL-8 receptor binds IL-8 with high affinity but growth-regulated α with low affinity, whereas the type B IL-8 receptor binds both IL-8 and growth-regulated α with high affinity. Hammond et al[20] used antibodies to assess the role of each receptor in the chemotactic response of neutrophils to growth-regulated α and found that growth-regulated α stimulates chemotaxis exclusively through type B receptors.

If growth-regulated α is involved in the pathogenesis of endometriosis, its level should be increased in the peritoneal fluid of women with endometriosis compared to normal women (controls); in addition, a greater increase should be seen with more severe disease. This is precisely the finding of this study. Growth-regulated α is found in greater quantities in women with endometriosis, and the more severe the disease the higher the growth-regulated α level.

If growth-regulated α is a participant in the pathogenetic pathway, where does this cytokine originate? Potential sources are macrophages, as well as mesothelial and endometrial cells in the peritoneal environment. We have found that cultured mesothelial cells constitutively express growth-regulated α messenger RNA and secrete growth-regulated α protein. Also, we found that cultured endometrial stromal cells express growth-regulated α messenger RNA and secrete growth-regulated α protein. Thus, the refluxed tissue may well represent a source of the growth-regulated α in endometriosis patients.

Finally, we asked how growth-regulated α secretion might be regulated. Our study presents evidence that the mesothelial and endometrial cell expression of this leukocyte chemoattractant is modulated by other cytokines such as IL-1α and TNF-α, cytokines that are produced mainly by activated macrophages and found in elevated levels in the peritoneal fluid of women with endometriosis. These cytokines appear to play a role in the constitutive secretion of growth-regulated α and are capable of greatly stimulating further production and secretion. Therefore, sources of IL-1 and TNF-α may play an important role in the initiation of the pathogenetic cascade: peritoneal mesothelium, endometrium, follicular fluid, and peritoneal macrophages are all candidate sources. Although there is no report on the production of TNF-α by peritoneal mesothelial cells, these cells do synthesize IL-1α and IL-1β.[21] There is also potential for an interplay between leukocytes and mesothelial cells: macrophages production of cytokines such as IL-1 and TNF-α may lead to increased production of growth-regulated α by mesothelial or endometrial cells in response.

In our study, the levels of growth-regulated α were significantly higher in the peritoneal fluid of women who had severe endometriosis than in women who did not have endometriosis, but levels were extremely low in women with endometriosis who had undergone

medical treatment with GnRH agonist. Taketani et al[22] have also observed that medical treatment of endometriosis decreases cytokine levels and concurrently eliminates the embryo toxicity of the peritoneal fluid in women with endometriosis. In addition, it has been reported[23] that treatment of endometriosis with medroxyprogesterone acetate reduces intraperitoneal inflammatory changes, which is exemplified by reduced peritoneal fluid volumes and lower leukocyte counts in the peritoneal fluid of those women.[23] Thus, growth-regulated α levels, like other inflammation markers, appear to decline with medical treatment of the disease.

On the basis of these findings, it is tempting to hypothesize that growth-regulated α is an active participant in the pathogenesis of endometriosis. In this report, we demonstrate that mesothelial and endometrial stromal cells can produce growth-regulated α constitutively and in response to IL-1α and TNF-α. Elevated levels of peritoneal growth-regulated α may play a role in the growth and maintenance of ectopic endometrial tissue, directly or indirectly stimulating endometrial cell proliferation. On the other hand, it is difficult to determine whether the increased growth-regulated α level in the peritoneal fluid is a cause or consequence of the disease because endometrial tissue itself produces growth-regulated α, and higher levels observed in advanced stages of endometriosis may be only reflecting the increased amount of endometrial tissue found in the peritoneal cavity of these patients. Future studies should be directed to discriminate between these two possible explanations.

References

1. Strathy JH, Molgaard CA, Coulman CB. Endometriosis and infertility: A laparoscopic study of endometriosis among fertile and infertile women. Fertil Steril 1982;38:667–72.
2. Olive D, Henderson D. Endometriosis and mullerian anomalies. Obstet Gynecol 1987;69:412–5.
3. Halme J, Hammond M, Hulka J, Raj S, Talbert L. Retrograde menstruation in healthy women and in patients with endometriosis. Obstet Gynecol 1984;64:151–4.
4. Halme J, Becker S, Haskill S. Altered maturation and function of peritoneal macrophages: Possible role in pathogenesis of endometriosis. Am J Obstet Gynecol 1987;156:783–9.
5. Fakih H, Bagget B, Holtz G, Tsang KY, Lee J, Williamson H. Interleukin-1: Possible role in the infertility associated with endometriosis. Fertil Steril 1987;47:213–7.
6. Halme J. Release of tumor necrosis factor-alpha by human peritoneal macrophages in vivo and in vitro. Am J Obstet Gynecol 1989;161:1718–25.
7. Oosterlynck DJ, Meuleman C, Waer M, Koninckx PR. Transforming growth factor-β activity is increased in peritoneal fluid from women with endometriosis. Obstet Gynecol 1994;83:287–92.
8. Leiva MC, Hasty LA, Pfeifer S, Mastroianni L Jr, Lyttle CR. Increased chemotactic activity of peritoneal fluid in patients with endometriosis. Am J Obstet Gynecol 1993;168:592–8.
9. Miller MD, Krangel MS. Biology and biochemistry of the chemokines: A family of chemotactic and inflammatory cytokines. Crit Rev Immunol 1992;12:17–46.
10. Arici A, Tazuke SI, Attar E, Kliman HJ, Olive DL. Interleukin-8 concentration in peritoneal fluid of patients with endometriosis and modulation of interleukin-8 expression in human mesothelial cells. Mol Hum Reprod 1996;2:40–5.
11. Sager R, Anisowicz A, Pike MC, Beckmann P, Smith T. Structural, regulatory, and functional studies of the GRO gene and protein. In: Baggiolini M, Sorg C, eds. Interleukin 8 (NAP-1) and related chemotactic cytokines. Basel (Switzerland): Karger, 1992:96–116.
12. Wen D, Rowland A, Derynck R. Expression and secretion of gro/MGSA by stimulated human endothelial cells. EMBO J 1989; 8:1761–6.
13. Haskill S, Peace A, Morris J, Sporn SA, Anisowicz A, Lee SW, et al. Identification of three related human GRO genes encoding cytokine functions. Proc Natl Acad Sci 1990;87:7732–6.
14. American Fertility Society. Revised American Fertility Society classification of endometriosis. Fertil Steril 1985;43:351–2.
15. Arici A, Head JR, MacDonald PC, Casey ML. Regulation of interleukin-8 gene expression in human endometrial cells in culture. Mol Cell Endocrinol 1993;94:195–204.
16. Richmond A, Balentien E, Thomas HG, Flaggs G, Barton DE, Spiess J, et al. Molecular characterization and chromosomal mapping of melanoma growth stimulatory activity, a growth factor structurally related to β-thromboglobulin. EMBO J 1988;7:2025–33.
17. Olive D, Montoya I, Riehl R, Schenken R. Macrophage-conditioned media enhance endometrial stromal cell proliferation in vitro. Am J Obstet Gynecol 1991;164:953–8.
18. Anisowicz A, Bardwell L, Sager R. Constitutive overexpression of a growth-regulated gene in transformed Chinese hamster and human cells. Proc Natl Acad Sci USA 1987;84:7188–92.
19. Moser B, Clark-Lewis R, Zwahlen R, Baggioloni M. Neutrophil-activating properties of the melanoma growth-stimulatory activity. J Exp Med 1990;171:1797–802.
20. Hammond MEW, Lapointe GR, Feucht PH, Hilt S, Gallegos CA, Gordon CA, et al. IL-8 induces neutrophil chemotaxis predominantly via type I IL-8 receptors. J Immunol 1995;155:1428–33.
21. Lanfrancone L, Boraschi D, Ghiara P, Falini B, Grignani F, Peri G, et al. Human peritoneal mesothelial cells produce many cytokines and are activated and stimulated to grow by IL-1. Blood 1992;80: 2835–42.
22. Taketani Y, Kuo TM, Mizuno M. Comparison of cytokine levels and embryo toxicity in peritoneal fluid in infertile women with untreated or treated endometriosis. Am J Obstet Gynecol 1992;167: 265–70.
23. Haney AF, Weinberg JB. Reduction of the intraperitoneal inflammation associated with endometriosis by treatment with medroxyprogesterone acetate. Am J Obstet Gynecol 1988;159:450–4.

Address reprint requests to:
Aydin Arici, MD
Department of Obstetrics and Gynecology
Yale University School of Medicine
333 Cedar St.
New Haven, CT 06520-8063

Received May 8, 1996.
Received in revised form August 12, 1996.
Accepted September 9, 1996.