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Monocyte chemotactic protein-1 concentration in peritoneal fluid of women with endometriosis and its modulation of expression in mesothelial cells*

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Objective: To investigate monocyte chemotactic protein-1 concentrations in the peritoneal fluid (PF) of women with or without endometriosis, then assess peritoneal mesothelial cells as a potential source of monocyte chemotactic protein-1.

Design: Prospective study.

Setting: University medical center.

Patient(s): Women with (n = 60) or without (n = 18) endometriosis.

Intervention(s): First monocyte chemotactic protein-1 levels in PF were measured, then mesothelial cells in culture were treated with cytokines.

Main Outcome Measure(s): In PF and culture supernatants, monocyte chemotactic protein-1 was measured by ELISA. In vitro monocyte chemotactic protein-1 messenger RNA expression was evaluated by Northern analysis.

Result(s): The median concentration of monocyte chemotactic protein-1 in PF of control women was 137 pg/mL (conversion factor to SI unit, 0.115; range, 12 to 418 pg/mL); that of women with moderate endometriosis was 205 pg/mL (range 65 to 6,000 pg/mL); and that of those with severe endometriosis was 1,165 pg/mL (0 to 2,602 pg/mL). Within the moderate to severe endometriosis group, monocyte chemotactic protein-1 levels were higher in women with untreated endometriosis (354 pg/mL range 0 to 6,000 pg/mL) than in women receiving GnRH agonist (128 pg/mL, range 0 to 216 pg/mL). In the control group, monocyte chemotactic protein-1 levels were higher in the proliferative phase than in the secretory phase. Mesothelial cells produced constitutively monocyte chemotactic protein-1; moreover, both interleukin-1α and tumor necrosis factor-α induced higher levels of monocyte chemotactic protein-1.

Conclusion(s): Levels of monocyte chemotactic protein-1 in PF were higher during the proliferative phase than secretory phase of control women and increased in moderate to severe endometriosis. The regulated expression of monocyte chemotactic protein-1 may recruit macrophages into PF and contribute to the pathogenesis of endometriosis. (Fertil Steril® 1997;67:1065–72. © 1997 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, monocyte chemotactic protein-1, peritoneal fluid, cytokines

Endometriosis is among the most common of gynecologic entities, with a prevalence estimated to be 9% to 33% (1) among women of reproductive age. In spite of more than 6 decades of intensive investigation, the pathogenesis of endometriosis is still poorly understood. Considerable circumstantial evidence supports the role of retrograde menstruation with subsequent endometrial implantation as an etiologic factor in most cases (2). Although retrograde men-

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struation is a nearly universal phenomenon among cycling women (3), it is not clear why endometrial tissue will implant and grow in the peritoneal cavity of only a subgroup of women.

In recent years, immunologic dysfunction has been associated with endometriosis. Peritoneal fluid (PF) is an immunologically dynamic environment that links the reproductive and immune system. Cells floating in the PF are mainly composed of macrophages. There is an increased number, concentration, and activation of macrophages in the PF of women with endometriosis (4). Secretory products of macrophages such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and growth factors are also found in increased quantity in the PF of these women (5). Finally, macrophage-conditioned media have been shown to enhance mouse endometrial stromal cell proliferation in vitro (6).

One of the primary abnormalities in women with endometriosis may be an enhanced recruitment of macrophages into the peritoneal cavity. There are several candidate chemoattractants that may recruit the macrophages. One of them is monocyte chemotactic protein-1, a specific factor that chemoattracts and activates monocytes and macrophages. Monocyte chemotactic protein-1 is secreted by a number of cell types including endothelial cells, fibroblasts (7), and leukocytes (8). Its expression is regulated by IL-1, TNF-α, platelet-derived growth factor (PDGF), and interferon-γ (IFN-γ) in endometrial cells in culture (9).

We hypothesized that such a monocyte chemotactic and activating factor may play an important role in the recruitment of macrophages to the peritoneal cavity and thus play a role in the pathogenesis of endometriosis. In the present study, we have investigated monocyte chemotactic protein-1 concentrations in the PF of women with or without endometriosis, then assessed peritoneal mesothelial cells as a potential source of PF monocyte chemotactic protein-1.

MATERIALS AND METHODS

Peritoneal Fluid Collection

Peritoneal fluid samples were obtained from women undergoing diagnostic laparoscopy. Indications for laparoscopy included evaluation of infertility, pelvic pain, pelvic mass, or elective tubal sterilization. Informed consent was obtained from each woman prior to surgery, using consent forms and protocols approved by the Human Investigation Committee of this University. The day of the menstrual cycle was established from the woman's menstrual history and verified in a subset by histologic examination of the endometrium. Patients were classified into five groups according to anatomic findings observed during the procedure: normal fertile patients (n = 18) (undergoing laparoscopic tubal ligation), and patients with minimal (n = 13), mild (n = 15), moderate (n = 24), and severe (n = 8) endometriosis as defined by the revised American Fertility Society classification (10). Patients (n = 5) with the diagnosis of moderate or severe endometriosis in whom the surgical procedure was performed as an evaluation of continuing medical treatment for the disease were also analyzed separately. This group of patients was receiving GnRH agonist (GnRH-a) (Lupron Depot; TAP Pharmaceuticals, North Chicago, IL) for at least 6 months before the surgery. After insertion of the laparoscope and ancillary trocars, PF was aspirated immediately to avoid blood contamination. Fluid from the anterior and posterior cul de sacs were collected into a sterile syringe and centrifuged at 600 $\times$ g for 10 minutes at 4°C to remove cells, aliquoted, and frozen at −80°C until assayed. Cell pellets were resuspended in HBSS for mesothelial cell culture as described below.

Mesothelial Cell Cultures

Peritoneal cell pellets that were resuspended in Hanks' balanced salt solution (HBSS) were layered over Ficoll-Paque (Pharmacia LKB, Piscataway, NJ) and centrifuged at 400 $\times$ g for 30 minutes, as described before (11). Cells at the interface were pelleted once in HBSS once then plated in plastic flasks (25 cm^2) and allowed to replicate to confluence in M-199 medium that contained fetal bovine serum (10%, vol/vol), antibiotics-antimycotics (1%, vol/vol), and epidermal growth factor (EGF, 20 ng/mL) at 37°C in a humidified atmosphere (5% CO_2 in air). Thereafter, the cells were passed by standard methods of trypsinization, plated in culture dishes (10-mm diameter or 24-well plates) as appropriate for the experimental design, and allowed to replicate to confluence before commencement of each experiment. Cultured human peritoneal mesothelial cells were flat and polygonal when confluent and grew with a doubling time of 7 to 12 days. The mesothelial cells grew and formed a homogenous population after three passages, whereas contaminating macrophages, because they were terminal cells, stopped dividing. All experiments were done using cells at third passage. Experiments were commenced 1 to 3 days after confluence was attained. Because we have shown previously (9) in endometrial stromal cells in culture that serum does have a direct stimulatory effect on the production of monocyte chemotactic protein-1, the confluent cells were treated with serum-free, EGF-free M-199 media for 24 hours before treatment with test agents was initiated. All experiments were conducted using the serum-free, EGF-

free media. At the end of each experiment, the culture media were collected and frozen at −80°C for quantification of monocyte chemotactic protein-1 by ELISA. Cells were used for quantification of total protein or for isolation of RNA.

Immunocytochemical Analyses of the Cultured Cells

Peritoneal cells in culture were characterized by use of immunocytochemistry. Cells were plated in six-well dishes containing flamed 22-mm^2 coverslips. At preconfluence, cells were washed with phosphate-buffered saline (PBS) (pH 7.4), fixed with Bouin's fixative for 10 minutes and then washed again with PBS. Using the avidin-biotin method (Vectastain ABC Kit; Vector Laboratories, Burlingame, CA), cells were stained with the following mouse monoclonal antibodies: low-molecular-weight cytokeratin (1.3 mg of immunoglobulin (Ig) G per mL; DAKO, Carpinteria, CA) as a marker of epithelial cells, vimentin (0.5 mg protein per mL; BioGenex, San Ramon, CA) as a marker of fibroblasts, and HAM56 (0.8 mg of IgG per mL; DAKO) as a marker of macrophages. Approximately 95% of the cells were positive for both vimentin and cytokeratin by parallel staining, a finding characteristic of mesothelial cells (12). Fewer than 1% of the cells were positive for HAM56 after two or three passages. We also pelleted cells from PF without culturing, fixed them, then immunohistochemically stained them as described above. The staining pattern seen in approximately 70% of freshly collected cells was consistent with that of mesothelium, a finding confirming earlier observations by other investigators (13, 14).

Preparation of Total RNA and Northern Analysis

Total RNA was prepared by the guanidinium isothiocyanate-cesium chloride ultracentrifugation method. 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 by use of UV light. Prehybridization was conducted for 5 hours at 42°C in buffer containing 5× standard saline citrate (SSC), 5× Denhardt's solution, formamide (50%, vol/vol), dextran sulfate (5%, wt/vol), NaH_2PO_4 (50 mmol), and salmon sperm DNA (0.5 mg/mL). Hybridizations were conducted for 16 hours at 42°C in buffer composed of 5× SSC, 2× Denhardt's solution, formamide (50%, vol/vol), dextran sulfate (10%, wt/vol), NaH_2PO_4 (20 mmol), and salmon sperm DNA (0.1 mg/mL) with a complementary DNA (cDNA) probe (5 to 15 μCi) complementary to MCP-1 messenger RNA (mRNA) radiolabeled with [α-^{32}P]-deoxycitidine 5′-triphosphate by random hexamer priming. This probe was kindly provided to us by Dr. T. Yoshimura (NCI, Frederick, MD). After hybridizing, the blots were washed with 1× SSC and sodium dodecyl sulfate (SDS) (0.1%, wt/vol) for 15 minutes at room temperature, once with 0.1× SSC and SDS (0.1%, wt/vol) for 15 minutes at room temperature, and once for 20 minutes at 65°C. Autoradiography of the membranes was performed at −70°C using Kodak X-Omat AR film (Eastman Kodak, Rochester, NY). The presence of unequal amounts of total RNA in each lane was adjusted for by visualization of ethidium bromide-stained 28S and 18S ribosomal RNA subunits and by analysis of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA, using a cDNA probe (Clontech Laboratories, Palo Alto, CA) radiolabeled with [α-^{32}P]deoxycitidine 5′-triphosphate by random hexamer priming. The autoradiographic bands were quantified by using a laser densitometer (Molecular Dynamics, Sunnyvale, CA). Each monocyte chemotactic protein-1 band was normalized by using the value for the corresponding G3PDH mRNA, thus correcting for any variation in amounts of RNA applied to each lane. Similar experiments were conducted on three different occasions with cells prepared from different PFs.

Monocyte Chemotactic Protein-1 Immunoassay

Immunoreactive monocyte chemotactic protein-1 in PF samples and culture supernatant was quantified using an ELISA (R&D Systems, Minneapolis, MN). According to the manufacturer, this assay has no measurable cross-reactivity with other known cytokines. The sensitivity for monocyte chemotactic protein-1 was 4.7 pg/mL of sample. All of the PF samples were evaluated in a duplicate assay. Validation of its use for human PF was also performed: recombinant monocyte chemotactic protein-1 was diluted in assay buffer and pooled PF, and parallelism was observed between the standard curve of buffer and PF dilutions. Each experiment was done using three replicate wells for each condition, and supernatant from each well was tested in a single ELISA assay. Each experimental setup was repeated on at least three occasions using mesothelial cells obtained from three different patients. The intra-assay and interassay coefficients of variation were 4.9% and 5.9%, respectively.

Statistical Analyses

Because the levels of monocyte chemotactic protein-1 in the PF were not normally distributed, they were analyzed using a nonparametric method to test the equality hypothesis against an ordered alternative and within group differences by post hoc analy-

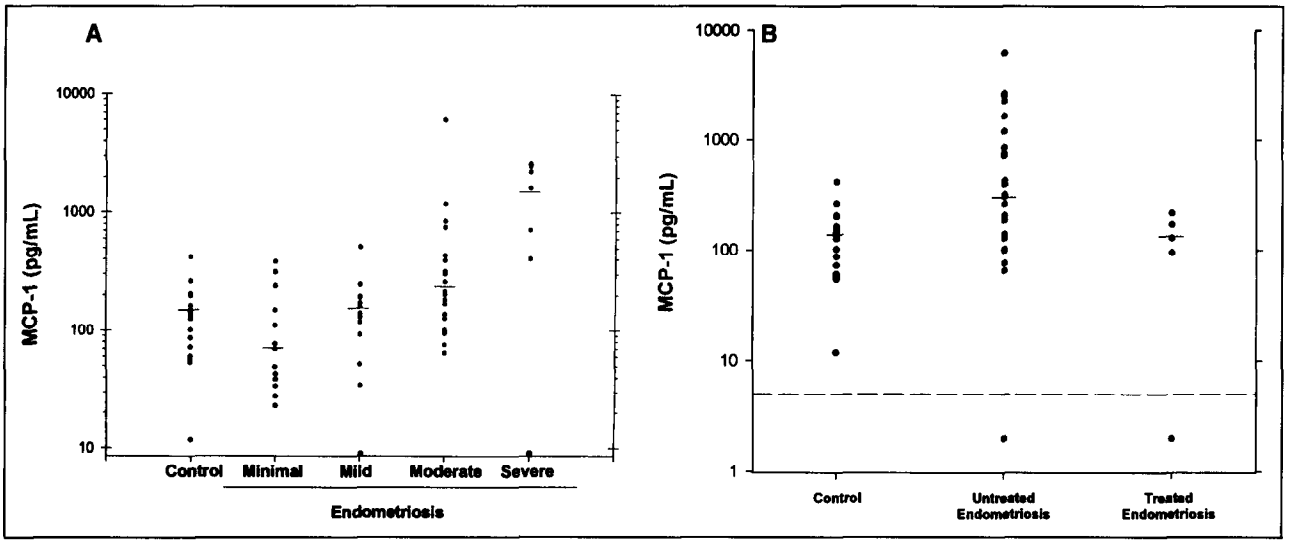

Figure 1 (**A**) Immunoreactive MCP-1 levels according to endometriosis stage: control (tubal ligation) (n = 18), minimal (n = 13), mild (n = 15), moderate (n = 24), and severe (n = 8) endometriosis. **Horizontal lines** represent medians. $P < 0.001$ overall; $P = 0.01$ for control versus moderate groups; $P = 0.03$ for control versus severe groups. (**B**) Concentration of MCP-1 in peritoneal fluid from women without endometriosis (n = 18), with untreated moderate or severe endometriosis (n = 26), and with medically (GnRH agonist) treated moderate or severe endometriosis (n = 5). *Horizontal lines* represent medians. *Dashed line* represents the lowest detection limit of the ELISA. $P = 0.03$ for treated versus untreated or control groups. MCP-1, monocyte chemotactic protein-1.

sis using the Mann-Whitney U test for multiple comparisons. Correlation analysis by ranks was calculated using the Spearman correlation coefficient. Data from ELISA assays of cell culture supernatants were normally distributed and evaluated by analysis of variance with Bonferroni post hoc analysis for multiple comparisons.

Reagents

Culture media, antibiotics–antimycotics, and fetal bovine serum were from Sigma Chemical Co. (St. Louis, MO). Epidermal growth factor, IL-1α, TNF-α, PDGF, IFN-γ (all recombinant), were from R&D Systems.

RESULTS

Immunoreactive Monocyte Chemotactic Protein-1 in Peritoneal Fluid

The median concentration of MCP-1 in PF samples obtained from control patients (n = 18) was 137 pg/mL (range, 12 to 418 pg/mL). In patients with endometriosis, we observed a stage-dependent elevation in the PF monocyte chemotactic protein-1 concentration. In PF samples obtained from patients with minimal endometriosis (n = 13) the median monocyte chemotactic protein-1 level was 70 pg/mL (range, 23 to 380 pg/mL); with mild endometriosis (n = 15), it was 135 pg/mL (range, 0 to 508 pg/mL); with moderate endometriosis (n = 24) the level was 205 pg/mL (range, 65 to 6,000 pg/mL); and with severe endometriosis (n = 8), it was 1,165 pg/mL (range, 0 to 2,602 pg/mL) (Fig. 1A). A significant difference among the five groups was observed ($P < 0.001$), with post hoc analysis revealing a difference between control versus moderate endometriosis ($P = 0.01$) and control versus severe endometriosis groups ($P = 0.03$). There was a significant correlation between the concentration of monocyte chemotactic protein-1 and the stage of endometriosis by rank correlation analysis ($r = 0.44$; $P = 0.001$). Within the moderate or severe endometriosis group, the levels of monocyte chemotactic protein-1 were significantly higher in the PF from women with untreated endometriosis (354 pg/mL (range, 0 to 6,000 pg/mL; n = 26) than in women who had undergone medical treatment with GnRH-a (128 pg/mL; range 0 to 216 pg/mL]; n = 5; $P = 0.03$) (Fig. 1B).

In women without endometriosis a menstrual cycle specific pattern was observed, with the concentration of monocyte chemotactic protein-1 in the proliferative phase (152 pg/mL [range, 133 to 206 pg/mL]; n = 5) being significantly greater than in the secretory phase (72 pg/mL [range, 12 to 114] pg/mL; n = 7; $P = 0.01$). The cyclic variation of monocyte chemotactic protein-1 in PF from women with endometriosis was not statistically different. The mean concentration of monocyte chemotactic protein-1 in the proliferative phase was 144 pg/mL (range, 34 to 2,236 pg/mL) (n = 19) and in the secretory phase

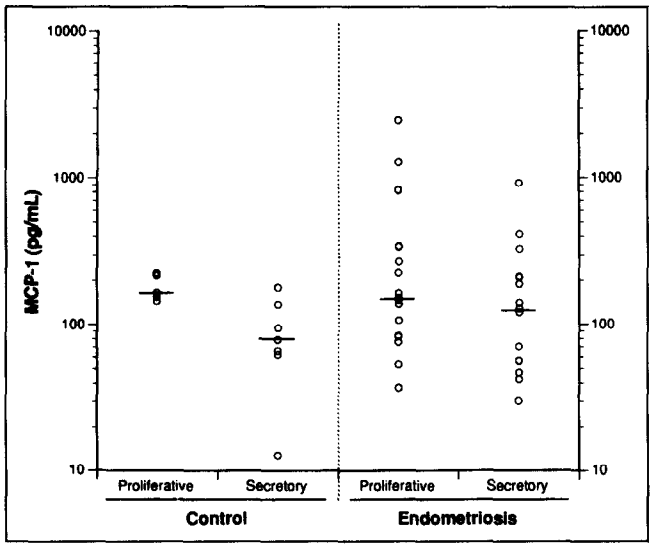

Figure 2 Menstrual cycle distribution of MCP-1 concentration in peritoneal fluid of women with or without endometriosis. *Horizontal lines* represent the medians; $P = 0.01$ for proliferative versus secretory phase in control group; $P = 0.1$ for proliferative versus secretory in endometriosis group. MCP-1, monocyte chemotactic protein-1.

was 118 pg/mL (range, 28 to 843 pg/mL) (n = 15) ($P = 0.1$) (Fig. 2).

Monocyte Chemotactic Protein-1 Production by Mesothelial Cells

Monocyte chemotactic protein-1 mRNA was detectable by Northern analysis in nontreated mesothelial cells. An increase in the levels of monocyte chemotactic protein-1 mRNA was seen when mesothelial cells were treated with increasing concentrations of IL-1α (range, 0.01 to 100 U/mL) and this effect was observable starting at the concentration of 0.1 U/mL (data not shown). Similarly a concentration-dependent increase in the levels of monocyte chemotactic protein-1 mRNA was observed with TNF-α (0.01 to 100 ng/mL), this increase was detectable starting at a concentration of 0.01 ng/mL (Fig. 3). In mesothelial cells treated with IL-1α (10 U/mL), the level of monocyte chemotactic protein-1 mRNA increased as early as 1 hour after the treatment and continued to rise for as long as 6 hours before declining (data not shown). Cotreatment of these cells with TNF-α (10 ng/mL) and PDGF (10 ng/mL) resulted in an additive effect on the induction monocyte chemotactic protein-1 mRNA, whereas IL-1α (10 U/mL) resulted in a synergistic increase of both TNF-α– and PDGF-induced monocyte chemotactic protein-1 mRNA (Fig. 4). Interferon-γ (500 U/mL) treatment of peritoneal mesothelial cells also resulted in a marked increase of monocyte chemotactic protein-1 mRNA levels in these cells (data not shown).

Nontreated mesothelial cells in culture produced and secreted monocyte chemotactic protein-1 protein quantified by ELISA. Treatment with IL-1α (10 U/mL) or TNF-α (10 ng/mL) caused increases in the accumulation of immunoreactive monocyte chemotactic protein-1 in the media; this difference was significant by 8 hours of treatment ($P = 0.001$). After 24 hours of IL-1α and TNF-α treatment, the longest duration of the experiments, maximal accumulation of monocyte chemotactic protein-1 in the media was observed ($P = 0.0001$) (Fig. 5). A similar, although lower, increase in the production of monocyte chemotactic protein-1 was also observed when cells were treated at lower concentration of IL-1α (1 U/mL) or TNF-α (1 ng/mL) (data not shown).

DISCUSSION

Although endometriosis is a relatively common gynecologic disorder, a lack of understanding of its pathogenesis has hindered research into prevention and treatment. Many theories for pathogenesis have surfaced over the years, but the mechanism appears to require endometrium and retrograde menstruation in most cases of the disease. This is consistent with epidemiologic data correlating a higher risk of the disorder with decreased parity, increased num-

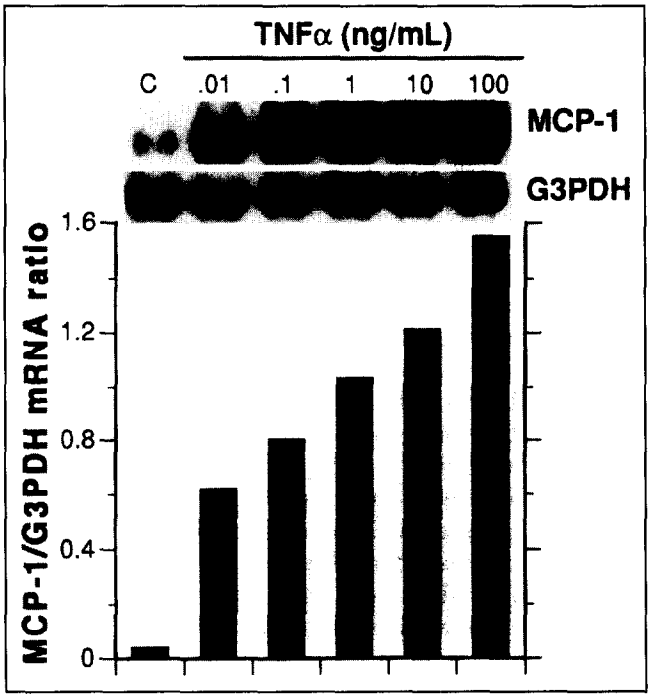

Figure 3 Northern analysis of MCP-1 mRNA in mesothelial cells treated with TNF-α: dose response. Confluent mesothelial cells in culture were incubated for 6 hours in serum-free culture medium with TNF-α (0.01 to 100 ng/mL). Total RNA (10 μg per lane) was evaluated. C, control; MCP-1, monocyte chemotactic protein-1.

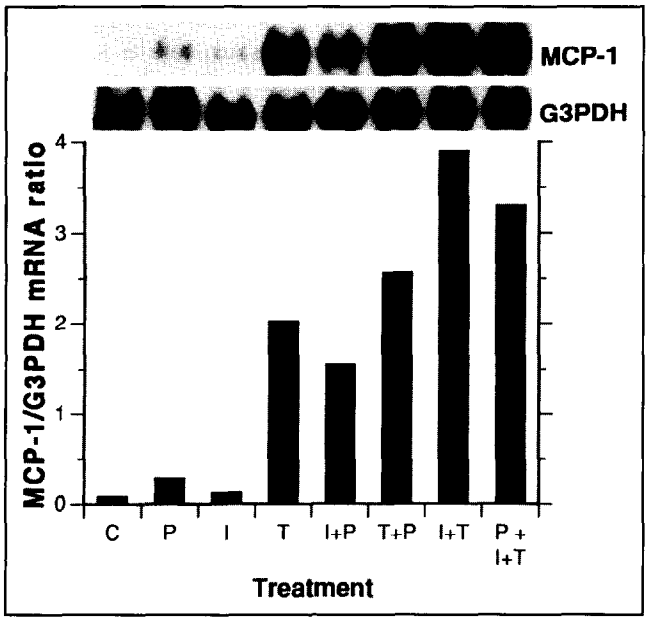

Figure 4 Northern analysis of monocyte chemotactic protein-1 mRNA in mesothelial cells treated with PDGF, IL-1α, and TNF-α. Confluent mesothelial cells in culture were incubated for 6 hours in serum-free culture medium with PDGF (10 ng/mL), IL-1α (10 U/mL), or TNF-α (10 ng/mL). Total RNA (10 μg per lane) was evaluated. MCP-1, monocyte chemotactic protein-1; C, control; I, IL-1α; T, TNF-α; P, PDGF.

ber of menses, early menarche, and heavier menstrual flow (15). In the baboon model, intraperitoneal placement of menstrual endometrium causes a higher rate of ectopic implantation than the rate seen with placement of endometrial fragments from the remainder of the menstrual cycle (16).

It thus appears that retrograde menstruation is a necessary condition for most endometriosis. It is also clear, however, that retrograde menstrual flow is a nearly universal phenomenon, whereas endometriosis is found in 10% to 30% of women of reproductive age. This suggests that other factors, such as the genetic predisposition, amount of retrograde flow, or immunologic changes, may determine a women's susceptibility to endometriosis. There is growing evidence that defects in the cellular immune system could be important for the pathogenesis of endometriosis. The environment of the peritoneal cavity may also play a decisive role in the development of endometriosis. Promoters within this peritoneal environment might well increase the likelihood of endometrial implantation and growth.

Peritoneal fluid of women with endometriosis has increased chemotactic activity for macrophages (17). The presence of such a chemotactic stimulus will increase macrophage number and activation, resulting in secretion of a variety of growth factors. Several investigators have demonstrated that endometrial stromal cell growth can be optimized in an environment of estrogen and growth factors, particularly by those growth factors secreted by peritoneal macrophages (6, 18). In addition, monocytes from women with endometriosis seem to enhance autologous endometrial cell proliferation, whereas monocytes from fertile women without endometriosis suppress endometrial cell proliferation (19). These findings suggest that an abnormality in monocyte or macrophage function may at the least, be supportive of ectopic endometrial growth and at the most, be causal for it.

There is compelling evidence suggesting that the PF in women with endometriosis is proinflammatory. Although this fact is now widely accepted, investigators have remained divided on whether these changes precede the disease or are a consequence of endometriosis. Whichever the case, it would seem critical to determine what factor(s) is responsible for the influx of macrophages. One of the candidates for peritoneal macrophage recruitment is monocyte chemotactic protein-1, a 76-amino acid basic protein that has chemotactic activity for monocytes. If monocyte chemotactic protein-1 is truly involved in the pathogenesis of endometriosis, the level of monocyte chemotactic protein-1 should be increased in the PF of women with endometriosis versus controls; in addition, a greater increase should be seen with more severe disease. This is precisely the finding of the present study. In a recent report, Akoum et al. (20) reported a significant elevation of monocyte chemotactic protein-1 levels in minimal to mild endometri-

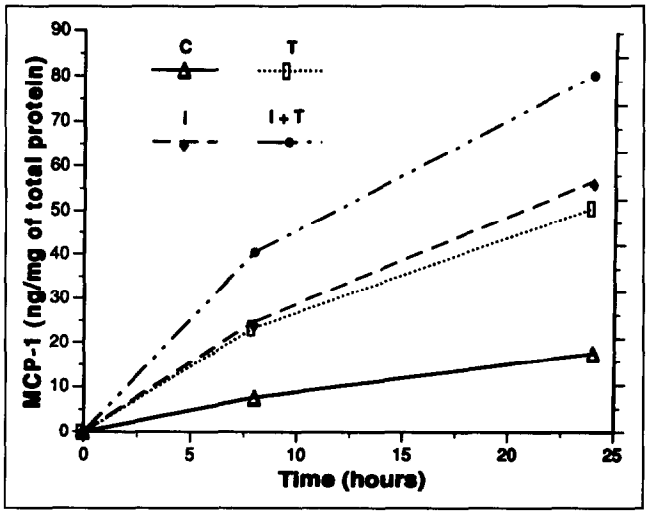

Figure 5 Stimulation of immunoreactive MCP-1 production by mesothelial cells in culture by IL-1α and TNF-α. Confluent mesothelial cells were treated with serum-free medium alone (control) or with medium containing IL-1α (10 U/mL) or TNF-α (10 ng/mL) or both for 8 and 24 hours. The culture media were collected, and MCP-1 was quantified by ELISA. Data are means ± SE for four replicates. MCP-1, monocyte chemotactic protein-1; C, control; I, IL-1α; T, TNF-α.

osis, but they did not observe significant elevation in the PF of women with moderate or severe endometriosis. The study, however, involved only four patients with these advanced stages, which limited the analysis of any correlation between PF concentrations of monocyte chemotactic protein-1 and the severity of the disease.

The level of monocyte chemotactic protein-1 was significantly higher in the PF from women who had severe endometriosis than from women who did not have endometriosis; but it was suppressed in women who had undergone medical treatment with GnRH-a. This finding may be because of suppression of endometriotic implants, with a resulting decrease in intraperitoneal inflammation. Taketani et al. (21) have observed that medical treatment of endometriosis decreases cytokine levels and eliminates the embryo toxicity of the PF from women with endometriosis. In addition, medroxyprogesterone acetate treatment of endometriosis reduces intraperitoneal inflammatory changes assessed by PF volume and leukocyte counts of the PF (22). Thus, the decrease in the monocyte chemotactic protein-1 levels associated with GnRH-a treatment would be consistent with a direct hormonal modulation of this cytokine. Our finding that monocyte chemotactic protein-1 levels in the PF of women without endometriosis is significantly higher in the proliferative phase than in the secretory phase also suggests a steroid hormone-dependent expression of monocyte chemotactic protein-1. Regulation of monocyte chemotactic protein-1 expression by steroid hormones in responsive tissues remains to be further elucidated.

If monocyte chemotactic protein-1 is a participant in the pathogenetic pathway of endometriosis, the next question is from where does this cytokine originate. Endometrial cells have been shown to produce monocyte chemotactic protein-1 (9). Thus, the refluxed tissue or associated fluid may well represent a source of the monocyte chemotactic protein-1 in endometriosis patients. Additionally, the implants themselves may represent a source of monocyte chemotactic protein-1. In a recent study, endometriotic cells have been shown to secrete monocyte chemotactic protein-1 in vitro upon stimulation with IL-1β and TNF-α (23). Macrophages are also known producers of monocyte chemotactic protein-1 (8). A final candidate for monocyte chemotactic protein-1 production is the peritoneal mesothelial cell. This study has shown that cultured mesothelial cells constitutively express monocyte chemotactic protein-1 mRNA and secrete monocyte chemotactic protein-1 protein. Thus, sources of monocyte chemotactic protein-1 are abundant in these women.

Mesothelial cells are the major cell population in the peritoneal lining and would be expected to regulate cell traffic and to trigger specific cellular functions of migrating cells, such as macrophages. This study presents evidence that the expression of this leukocyte chemoattractant is modulated by other cytokines such as IL-1 and TNF-α in mesothelial cells. Sources of IL-1 and TNF-α may, therefore, play an important role in the initiation of the pathogenic cascade; peritoneal mesothelium, endometrium, follicular fluid (24), and peritoneal macrophages are all candidate sources. To our knowledge, although there is no report on the production of TNF-α by peritoneal mesothelial cells, these cells synthesize IL-1α and IL-1β (25). There is also potential for an interplay between leukocytes and mesothelial cells: macrophages, by producing cytokines such as IL-1 and TNF-α, may in turn stimulate production of monocyte chemotactic protein-1 by mesothelial cells. In vitro concentrations of IL-1 and TNF-α used in the experiments presented here are comparable with the in vivo levels observed in the PF of women with endometriosis (21). These findings suggest that human peritoneal mesothelial cells may well be involved in the regulation of inflammation as well as tissue regeneration processes.

On the basis of these findings, it is tempting to hypothesize that monocyte chemotactic protein-1 is an active participant in the pathogenesis of endometriosis. Elevated levels of peritoneal monocyte chemotactic protein-1 may play a role in the growth and maintenance of ectopic endometrial tissue by not only stimulating macrophages to secrete growth factors and cytokines, but also by directly stimulating endometrial cell proliferation. Our observation that PF monocyte chemotactic protein-1 levels are higher in the proliferative phase than in the secretory phase suggests that monocyte chemotactic protein-1 may play a direct or indirect role in endometrial cell proliferation. On the other hand, whether the increased monocyte chemotactic protein-1 level in the PF is a cause or consequence of the disease cannot yet be answered. Future studies should be directed at discriminating between these two possible scenarios.

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