Chemotaxis of macrophages by a peritoneal fluid protein in women with endometriosis*

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Objective: To expand on a preliminary study comparing the chemotactic potential of peritoneal fluid (PF) from women with and without endometriosis and to characterize this activity via immunosuppressants and a protease.

Design: Case control study.

Setting: University center.

Patient(s): Fifty-nine women with endometriosis and 44 without, undergoing laparoscopy.

Intervention(s): Collection of PF, endometriotic, ovarian, and endometrial biopsies at laparoscopy.

Main Outcome Measure(s): Chemotactic activity of PF was tested via an in vitro assay alone and in the presence of immunosuppressants cyclosporin A (CSA), FK506, rapamycin, and type XVII-b(S-V8) protease and in media incubated with endometriotic, ovarian, or endometrial biopsy specimens.

Result(s): The PF from women with endometriosis had significantly greater chemotactic activity (cells per well, mean ± SD) than without endometriosis (142 ± 39 versus 48 ± 17). Cyclosporin A significantly inhibited the chemotactic activity of the endometriotic PF; FK506 and rapamycin did not. Incubation of media with endometriotic tissue, but not ovarian or endometrial, for > 7 hours displayed chemotactic activity. Protease type XVII-b(S-V8) added to endometriotic PF inhibited this chemotactic activity.

Conclusion(s): Peritoneal fluid from patients with endometriosis contains a protein chemotactic factor attracting inflammatory cells into the peritoneal cavity, possibly secreted by endometriotic implants. This chemotactic factor may be a member of the immunophilin family because of its inhibition profile. (Fertil Steril 1997;67:865-9. © 1997 by American Society for Reproductive Medicine.)

Key Words: Chemotaxis, endometriosis, cyclosporin A

Many aspects of peritoneal fluid (PF) have been studied in the hope of identifying factors associated with the development and growth of endometriosis as well as the accompanying infertility.

A consistent finding in the PF of patients with endometriosis has been the increased number and activation status of macrophages (1–3). These acti-
that may be responsible for the initiation of this destructive cycle. We then sought to characterize this chemotactic factor as to secretion and inhibition.

**MATERIALS AND METHODS**

This study was conducted at the University of Pennsylvania Hospital, a large tertiary care medical center. The study protocol was approved by the University of Pennsylvania Investigational Committee on the Study of Human Beings.

Peritoneal fluid was collected from the posterior cul de sac of 103 women undergoing laparoscopy between February 1992 and June 1994. Patients were divided into two main categories: group I, fertile controls, no endometriosis (n = 44) and group II, patients with infertility or pelvic pain and the finding of endometriosis (n = 59). Group II was subdivided into three sections according to the American Society for Reproductive Medicine classification of endometriosis (16): stage I-II (n = 54), stage III (n = 3), and stage IV (n = 2). The aspirate was collected into a sterile syringe before any intervention, placed on ice, and centrifuged at 2,000 × g for 10 minutes. The supernatant was aliquoted into 3 mL vials and stored at −70°C (−94°F).

The ability of the samples to attract macrophages was measured by a quantitative assay developed by Boyd et al. in 1962 (17). The chemotactic assay was performed by means of a 48-well chemotactic chamber (Neuroprobe, Cabin John, MD) with a 250-μm pore polycarbonate filter (Neuroprobe), as has previously been described (15). Samples (30 μL) were placed in the lower chamber wells and incubated at 37°C (98.6°F) in humidified 5% carbon dioxide.

RESULTS

The PF from patients with endometriosis, group II, had significantly increased chemotactic activity (149 ± 39, mean ± SD) over the PF of patients without endometriosis, group I, 48 ± 17, P < 0.001) and over the negative controls (25 ± 12, P < 0.001) (Fig. 1). Three of the 44 samples in group I displayed chemotactic activity comparable with that of group II or positive controls. All of the samples in group II displayed chemotactic activity similar to FMLP (10), a known chemotactic peptide, which served as a positive control. The chemotactic activity of group II decreased as the stage of the disease advanced, with the greatest activity in stages I and II (mean = 153), stage II (mean = 93), and stage IV (mean = 75) (Fig. 1). However, because of the small sample size for stages III and IV, this was seen as a trend and more patients would be needed to reach statistical significance.

The data presented in Figure 2 denote the chemotactic activity of the media after incubation with endometriotic, ovarian, or endometrial tissue. The media progressively increased its chemotactic activity with the length of exposure time to endometriotic
Cells per Wall

Figure 1. Chemotactic activity of PF from patients with endometriosis. The chemotactic activity present in the PFs at different stages of disease was determined as described in Materials and Methods. FMLP served as a positive control and buffer (10 mM Tris-HCl pH 7.4) as a negative control.

Figure 2. Chemotactic activity of incubation media. Endometriotic, ovarian, and endometrial tissues were incubated as described in Materials and Methods, and samples of the incubation media were analyzed for chemotactic activity after 3, 5, 6, 7, and 18 hours of incubation.

Figure 3. Effect of immunosuppressants on chemotactic activity. Peritoneal fluid from individual patients with endometriosis (n = 15) was measured in the absence of or in the presence of 10 nM, 100 nM, or 1 μM cyclosporin A (CSA) and either FK506 or rapamycin at concentrations of 100 nM, 10 μM, or 1 mM.

Vol. 67, No. 5, May 1997

Weil et al. Chemotaxis in patients with endometriosis 867

A significant body of information has been assembled concerning the contents, volume, and characteristics of PF in patients with endometriosis (8, 20). Both leukocytes and macrophages have been studied in this context. Halme et al. (3) and Chacho et al. (8) have published concurring reports on an increase in macrophage content in the PF of women with endometriosis. Furthermore, both groups also recognized an increase in the activation status of these tissue. In contrast, media incubated with ovarian or endometrial tissue did not have significant chemotactic activity at any time interval.

The addition of type XVII-b (S-V8) protease to the PF completely inhibited the chemotactic properties in that of patients with endometriosis. The chemotactic activity (cells per well, mean ± SD) of FMLP alone, with 1 or 10 U protease measured 30 ± 7, 33 ± 6, and 34 ± 10, whereas, the chemotactic activity of the PF of patients with endometriosis alone, with 1 or 10 U of protease measured 35 ± 6, 9 ± 5, and 11 ± 4, for stage I-II, stage III, and stage IV, respectively, P < 0.001.

A previous study from our laboratory demonstrated that PF from patients with endometriosis revealed a chemotactic factor with a molecular weight of approximately 20 kd (15). In other models, we and others have shown that cyclophilin, a low-molecular-weight protein, demonstrated immunosuppressant inhibited chemotactic activity (19). Therefore, we investigated the ability of immunosuppressants, such as CSA, to modulate chemotactic activity found in the PF of patients with endometriosis. Addition of CSA to the PF samples in group II (endometriosis), significantly decreased the chemotactic activity using a concentration of CSA from 10 nM to 1 μM (P < 0.05) (Fig. 3). No effect was seen in group I (no endometriosis) after treatment with various concentrations of CSA.

In an attempt to examine the specificity of this inhibition by immunosuppressants, we tested the ability of both FK506 and rapamycin to inhibit this response. The addition of FK506 and rapamycin to the PF of group I and group II in concentrations of 100 nM and 10 μM did not significantly alter chemotactic activity (P > 0.05) (Fig. 3).

DISCUSSION

A significant body of information has been assembled concerning the contents, volume, and characteristics of PF in patients with endometriosis (8, 20). Both leukocytes and macrophages have been studied in this context. Halme et al. (3) and Chacho et al. (8) have published concurring reports on an increase in macrophage content in the PF of women with endometriosis. Furthermore, both groups also recognized an increase in the activation status of these...
Macrophones (3, 8). Activation of macrophages involves changes in size, rate of spread, adherence, rate, and extent of phagocytosis, plasma membrane enzymes, oxygen consumption, prostaglandin release, and increased secretory products (21).

In our hypothesis, we saw this increase in the number and activation status of peritoneal macrophages as a key step in the destructive cycle of endometriosis. A self-generating, cyclical course develops in which an increased number of activated macrophages are concentrated in the PF, which in turn produce interleukin 8 (IL-8) and tissue necrosis factor alpha (TNFα) (22). These cytokines then attract additional monocytes and inflammatory cells that create tissue fibrosis, formation of adhesions, and alteration of the normal pelvic anatomy. Therefore, we began by asking if the PF from patients with endometriosis when compared with control patients was attracting macrophages. In a previous publication, we demonstrated increased chemotactic activity of the PF in endometriosis (15). In our present study, extended to 108 patients, this finding is significantly reinforced. Hill et al. (1) found the peritoneal leukocyte concentration to be the highest in stage I endometriosis versus stage IV. This coincides with our finding of stage I-II endometriosis possessing the greatest chemotactic activity. As mentioned before, because of our small sample size in stage III and IV this finding needs confirmation. Such studies are underway.

Next, we wanted to test the possibility that the endometriotic tissue secretes a substance(s) that is a chemoattractant. In our study, the media in which endometriotic implants had been cultured displayed chemotactic activity within 18 hours, supporting this hypothesis. Ovarian and endometrial tissue did not change the activity of the incubated media. These data suggest that endometriotic tissue represents the source of the macrophage chemotactic activity. Because of the limited sample size, further investigation is required.

In an effort to identify this chemotaxis factor, protease addition was performed, which inhibited the chemotactic activity, suggesting that this factor is a protein that loses this chemotactic property as a result of cleavage.

Macrophages have specific receptors for chemoattractants that increase in number when these cells become activated (23). Immunophilins are a family of proteins that have been shown to demonstrate chemoattractive properties and are known to bind immunosuppressants. Cyclophilin, a member of this family, was discovered from bovine spleen in 1984 and is in the size range (20 kd) that we previously identified in the PF of patients with endometriosis (15, 23). This protein specifically binds CSA causing a three-dimensional structural change inhibiting its activity, including its chemotactic properties (23). As demonstrated by the studies presented here of the immunosuppressants examined, only CSA was able to inhibit the chemotactic activity of PF. The ability to prevent chemotaxis could, thus, halt the initiation of a destructive inflammatory environment in the pelvis of patients with endometriosis. Although fluid with higher concentrations of FK506 and rapamycin numerically had less activity, this did not reach statistical significance and may have been a dilutional effect (Fig. 3).

Recent findings by Atrici et al. (Atrici A, Attar E, Tazuke S, Oral E, Olive DJ, abstract) and Akoum et al. (25) have found increase levels of monocyte chemotactic factor 1 (MCP-1) in the PF of patients with endometriosis. MCP-1 is secreted by activated macrophages and by stimulated epithelial cells. The question arises, therefore, whether in the previously mentioned destructive cycle of monocytes and cytokines increased levels of MCP-1 in patients with endometriosis are a cause of initial macrophage migration or are a result thereof? MCP-1 is a 9-, 13-, or 15-kd protein; the protein presented here, as described previously, is larger (20-kd) (19). Antibody blockade of MCP-1 decreased chemotaxis of macrophages by 40% (24). We conclude from our data that endometriosis is secreting at least one other chemotactic protein completely inhibited by CSA.

This raises a host of questions for future research. It suggests that the chemotactic factor may be related to the cyclophilin family of proteins. It further suggests that discovery of an inhibitor of this activity may have utility in the treatment of endometriosis. Inhibition of excess inflammatory cell migration to endometriosis and the pelvic cavity could potentially decrease the destructive and painful effects endometriosis can produce.

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REFERENCES


