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Interleukin-8 concentrations are elevated in peritoneal fluid of women with endometriosis*

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Objective: To investigate the presence of interleukin-8 (IL-8), a macrophage-derived angiogenic factor, in peritoneal fluid (PF) of women with and without endometriosis.

Design: Case-control study.

Setting: University hospital.

Patients: Eighteen women with laparoscopic findings of mild to severe endometriosis, and nine women with no visual evidence of pelvic pathology.

Main Outcome Measures: Peritoneal fluid IL-8 levels were determined using an ELISA. Interleukin-8 concentrations were compared among women with and without endometriosis. Correlation between PF IL-8 concentration and endometriosis stage was investigated.

Results: Interleukin-8 was detectable in the PF of a majority of women (67%). Interleukin-8 concentrations were higher in the PF of women with endometriosis than in matched normal controls. A significant correlation between PF IL-8 concentration and endometriosis stage was noted.

Conclusions: We hypothesize that IL-8 is an important angiogenic factor that contributes to the pathogenesis of endometriosis by promoting the neovascularization of ectopic endometrial implants. Fertil Steril 1995;63:929–32

Key Words: Endometriosis, IL-8, peritoneal fluid, angiogenesis

Ectopic pelvic implants of endometrial glands and stroma can manifest a variety of visual patterns. One common observation is the presence of neovascularization around and within endometriosis implants (1). This observation suggests that angiogenesis may be an important step in the establishment and growth of pelvic endometriosis.

It recently has been shown that peritoneal fluid (PF) of women with endometriosis contains soluble factors with angiogenic activity (2). Although these factors have not been characterized yet, several angiogenic cytokines are potential candidates. One such cytokine is interleukin-8 (IL-8), an 8-kd protein derived from activated macrophages. In addition to its recently characterized function as a potent angiogenic factor, it is a chemoattractant for neutrophils and induces expression of several cell-adhesion molecules (3).

In this case-control study we investigated the presence of IL-8 in PF. Next we examined if differences in PF IL-8 concentrations could distinguish women with and without endometriosis. Finally, we

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sought evidence of a correlation between PF IL-8 concentration and severity of endometriosis.

MATERIALS AND METHODS

Women scheduled for laparoscopy, with normal menstrual cycles and not taking contraceptive steroids or GnRH analogs, were recruited into the study after providing informed consent under a protocol approved by the University of California, San Francisco Committee on Human Research. Indications for laparoscopy included evaluation of infertility, pelvic pain, pelvic mass, or elective tubal sterilization. The pelvis was assessed for the presence or absence of endometriosis and staged according to a modification of the revised American Fertility Society (4) staging system in which only active endometriosis lesions, but not adhesions, were scored. Women with visible lesions were assigned to stage I to II or stage III to IV endometriosis groups on the basis of active disease volume. Control subjects were women in whom no visible evidence of pelvic pathology was found. Nine women without evidence of pelvic pathology were age matched to nine women with stage I to II and nine women with stage III to IV endometriosis. Accurate menstrual cycle dating at the time of surgery was available for 22 of 27 (81%) subjects.

Laparoscopy was performed using the Verres needle technique. We controlled for the volume of saline (<1 mL) injected for confirmation of intraperitoneal needle placement. Peritoneal fluid was aspirated immediately on entering the coelomic cavity and transported to the laboratory on ice. Volumes of the specimens were recorded, and cells were removed from the fluids by centrifugation at 700 × *g* at 4°C for 10 minutes. The supernatant fractions were coded, aliquoted, placed in tubes containing aprotinin (1 μg/mL PF), and stored at −70°C until assayed.

Peritoneal fluid IL-8 concentrations were measured blindly, in duplicate, by a specific ELISA (R&D Systems, Minneapolis, MN). The sensitivity of the assay in PF was 10 pg/mL (conversion factor to SI units, 0.125) and was linear over a concentration range of 10 to 6,000 pg/mL. The coefficients of variation were <10%.

The cytokine data were not distributed normally, therefore, comparisons among the three study groups were analyzed conservatively using nonparametric analysis of variance by ranks (Kruskal-Wallis test) and within group differences by post hoc analysis using Mann-Whitney tests with Bonferroni correction for multiple comparisons. Correlation analysis by ranks (Spearman test) was performed also.

Interleukin-8 concentrations were compared with the phase of the menstrual cycle at time of sample collection in the 22 subjects for whom accurate cycle data were available, using the Mann-Whitney test. Each study group also was compared to menstrual cycle phase distribution at the time of sample collection, using contingency coefficients.

For the purpose of descriptive statistics (mean ± SD) we assumed an undetectable IL-8 level (<10 pg/mL) to be equal to 0 pg/mL. However, for inferential nonparametric statistics (Kruskal-Wallis, Spearman tests), ordinal scale data were used in which the former assumption is not made. Statistical significance was accepted at $P < 0.05$ for two-tailed analyses.

RESULTS

Interleukin-8 was detectable in the PF of 67% of cycling women (18 of 27 subjects). Interleukin-8 concentrations varied considerably among patients and their distribution according to endometriosis stage is shown in Figure 1. Interleukin-8 concentrations were higher in PF of women with endometriosis than in matched normal controls (Table 1). A significant difference among the three groups was

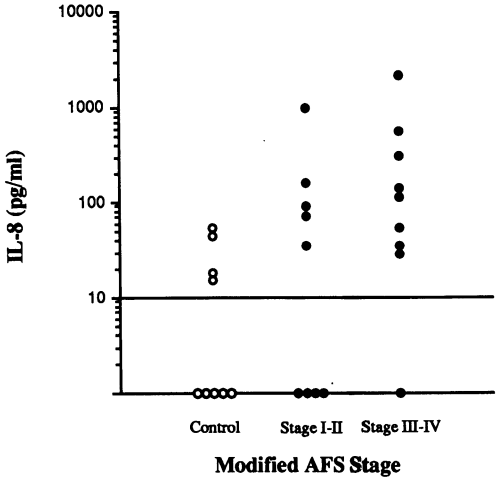

Figure 1 Distribution of IL-8 concentrations in PF of individual subjects from control and endometriosis study groups. The horizontal line represents the lower limit of sensitivity of the ELISA in PF (10 pg/mL).

Table 1 Clinical Characteristics and Peritoneal Fluid IL-8 Concentrations in Control and Endometriosis Study Groups*

	Study groups (n = 9)				
	Control	Endometriosis stage I to II	Endometriosis stage III to IV	Kruskal-Wallis H score	*P*
Age	32 ± 9	34 ± 6	30 ± 6	1.9	0.38
Parity	1.3 ± 2.7	0.8 ± 1.2	0.2 ± 0.6	1.1	0.60
Peritoneal fluid volume (mL)	17 ± 21	10 ± 3	15 ± 15	2.0	0.58
IL-8 concentration (pg/mL)†‡	15 ± 21 (0)	152 ± 317 (36)	380 ± 696 (115)	6.8	0.03
Total IL-8 (pg)†	133 ± 150 (91)	392 ± 515 (229)§	10,090 ± 24,271 (858)§	8.2	0.02

* Values are means ± SD.
† Values in parentheses are median concentrations.
‡ Conversion factor to SI unit, 0.125.
§ Significantly different between control and endometriosis stage III to IV, ($P < 0.02$) using post hoc Mann-Whitney test with Bonferroni correction.

observed ($P = 0.03$), with post hoc analysis revealing a difference between the control and stage III to IV endometriosis groups ($P < 0.02$). A significant correlation between PF IL-8 concentration and endometriosis stage also was noted ($r = 0.51$, $P < 0.01$).

The clinical characteristics of the subjects showed no differences in parity ($P = 0.60$) or in total PF volumes among the three study groups ($P = 0.58$) (Table 1). Follicular phase PF volumes (10.9 ± 2.1 mL, n = 16; mean ± SD) were not different from luteal phase fluid volumes (9.5 ± 3.0 mL, n = 6; $P = 0.48$). Within each study group there also was no significant correlation between IL-8 concentrations and cycle phase. Furthermore, the observed frequency of sample collection in the follicular (73%) versus the luteal (27%) phase of the cycle was identical in all three study groups ($c^2 = 0.03$, $P = 0.98$, n = 22).

DISCUSSION

This study shows that IL-8 levels are variable but detectable in the PF of most normally cycling women. We demonstrate that PF concentrations of IL-8 in women with endometriosis exceed those in normal controls and that IL-8 levels correlate with the volume of endometriosis implants. Using conservative nonparametric statistical analyses, a significant difference was shown between the control and endometriosis stage III to IV groups. Failure to detect a significant difference between the control and endometriosis stage I to II groups may be due to the limited statistical power in this series of patients. Analyses of PF volume and IL-8 concentration data in our study failed to identify significant effects of cycle phase on either of these two parameters. Thus, menstrual cycle effects on IL-8 concentrations in the PF do not provide an explanation for our findings. Whether the wide variation in IL-8 concentrations among women with endometriosis is related to the variable appearances of endometriotic lesions (1) was not addressed in our study.

Cellular components of the PF milieu have been shown to differ in women with endometriosis, including an increased number and activated state of macrophages. The hypothesis that endometriosis implants secrete cytokines that recruit and activate peritoneal macrophages has been proposed. Macrophage chemotactic activity is present in the PF of women with endometriosis. One of these chemokines, RANTES, has been shown to be present in higher concentrations in the PF of women with endometriosis than in normal controls (5). Activated peritoneal macrophages could be an important source of PF IL-8.

In vivo studies of angiogenesis reveal that activated macrophages and macrophage-derived cytokines play a critical role in the induction of neovascularization. Extending the tumor angiogenesis analogy to endometriosis implant neovascularization, we postulate that an implant could not grow greater than a few cubic millimeters unless new blood vessels were to sprout and to vascularize the implant. A vascularized implant would be expected to proliferate and to infiltrate into surrounding peritoneal tissues, as is commonly observed in active endometriosis.

In summary, our study shows that IL-8 is present in increased amounts in the PF of women with endometriosis and that its concentration correlates with implant volume. We hypothesize that IL-8 is an important macrophage-derived angiogenic fac-

tor that contributes to the pathogenesis of endometriosis by promoting the neovascularization and, hence, proliferation of ectopic endometrial implants.

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Note. Additional references are available upon request.