Peritoneal Fluid and Plasma Levels of Human Macrophage Colony-Stimulating Factor in Relation to Peritoneal Fluid Macrophage Content


The peritoneal fluid (PF) of women with infertility (especially in the presence of endometriosis) contains increased numbers of leukocytes, 90% to 95% of which are macrophages. The high numbers of peritoneal macrophages presumably result from an influx of blood monocytes into the peritoneum, and/or from local proliferation of peritoneal macrophages. Once in the peritoneal cavity, monocytes differentiate into tissue macrophages. Mononuclear phagocyte proliferation and differentiation are influenced by different cytokines, including macrophage colony-stimulating factor (M-CSF). The purpose of this study was to determine the relationship of M-CSF levels in human PF and plasma to the macrophage content, and to the patient diagnoses. Mean concentrations of PF M-CSF were higher than plasma levels (2.44 ± 0.13 vs 0.95 ± 0.06 ng/mL, respectively). The mean concentrations of plasma M-CSF did not differ in samples from women of different diagnostic groups (normal, peritoneal adhesions, endometriosis, inactive pelvic inflammatory disease, uterine fibroids, and idiopathic infertility), but the PF concentration was slightly higher in normal women. The absolute (total) amount of PF M-CSF in normal women was lower than in those of the other diagnostic groups. The total amount of PF M-CSF in all women correlated closely with the total number of peritoneal macrophages. The tubal patency status (open versus closed) did not influence the plasma and PF concentrations of M-CSF, nor the PF absolute amount of M-CSF. The PF M-CSF may have come from peritoneal macrophages, fibroblasts, mesothelial cells, or endothelial cells. PF M-CSF may play important roles in the proliferation and/or the differentiation of peritoneal mononuclear phagocytes.

WOMEN WHO ARE INFERTILE for a variety of reasons have increased volumes of peritoneal fluid (PF) and increased numbers of peritoneal macrophages.\(^1\)\(^3\) The increased number of peritoneal macrophages results from the influx of monocytes into the peritoneum, and/or from local proliferation of peritoneal macrophages. Mononuclear phagocyte proliferation is controlled by macrophage and granulocyte-macrophage colony-stimulating factor (M-CSF and GM-CSF), and interleukin-3 (IL-3).\(^1\) M-CSF is the primary growth factor for macrophages. It is produced by a variety of normal cells (mononuclear phagocytes, fibroblasts, endothelial cells, and some epithelial cells)\(^5\) and by certain tumor cells.\(^6\) In addition to the proliferative effects, M-CSF can cause differentiation or “activation” of monocytes and macrophages.\(^7\)\(^8\) The proliferative and differentiative effects of M-CSF are mediated by high-affinity membrane receptors.\(^4\)\(^5\) The purpose of this study was to determine the relationship of M-CSF levels in human PF and plasma to the macrophage content, and to the patient diagnoses. Results demonstrate that PF M-CSF levels were always higher than plasma levels, and that PF and plasma M-CSF concentrations did not correlate with PF volume, PF macrophage concentration or absolute number, or tubal patency status (open or closed). However, the absolute (total) amount of PF M-CSF correlated closely with the absolute number (total) of PF macrophages.

MATERIALS AND METHODS

After appropriate informed consent, matched sets of PF and blood were obtained from women undergoing laparoscopy for elective sterilization or investigation/treatment for infertility. The samples were anticoagulated with 10 U/mL of heparin, 0.3% EDTA, and 0.2 mol/L epsilon amino caproic acid. PF was obtained via a laparoscope as described before.\(^7\) The patient was positioned so that the fluid pooled in the dependent aspect of the peritoneal cavity and all of the free PF was collected; no rinsing of the peritoneal cavity was performed. Care was taken to avoid contaminating the PF with blood. Greater than 90% of the peritoneal cells were macrophages (nonspecific esterase [alpha naphthyl butyrate esterase]-positive) and all were viable (trypan blue exclusion). Samples were taken between days 10 and 20 of the menstrual cycle.

The patients were not taking any medications. Diagnoses were established by standard means.\(^1\)\(^2\) Samples from 67 subjects were studied. There were six normal subjects (those for elective bilateral tubal ligation [BTL] or tubal reanastomosis), two with idiopathic peritoneal adhesions, 32 with endometriosis, 16 with inactive pelvic inflammatory disease (PID), five with uterine fibroids, and six with infertility. M-CSF was measured with a radioimmunoassay using a highly specific rabbit anti-M-CSF antibody that does not cross-react with other growth factors.\(^9\) This assay is capable of detecting levels ≥0.5 ng/mL. Statistical analyses were performed using the “Statview” program (Abacus Concepts, Berkeley, CA) with the Student’s t-test and Mann-Whitney U test.

RESULTS

In all sets of PF and plasma examined, the mean PF M-CSF level was higher than the mean plasma level (2.44 ± 0.13 [mean ± SEM] vs 0.95 ± 0.06 ng/mL for PF and plasma, respectively [n = 67]; P < .0005), with PF levels higher than the corresponding plasma levels in all individual cases. The concentrations of M-CSF in plasma did not differ among the various diagnostic groups, but the PF concentrations were slightly higher in normal women undergoing bilateral tubal ligation (Fig 1A and B). PF volumes varied considerably among the groups, with the

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Fig 1. Plasma (A) and PF (B) M-CSF concentrations in women with different diagnoses. The bars show the means ± SEM. Statistical comparisons among the different groups showed significant differences between values for BTL versus endometriosis ($P < .005$), BTL versus fibroids ($P < .02$), and BTL versus PID ($P < .005$).

Fig 2. Total PF M-CSF levels (concentration times volume) in women with different diagnoses. The bars show the means ± SEM. Statistical comparisons among the different groups showed significant differences between values for BTL versus endometriosis ($P < .05$), and BTL and reanastomosis versus endometriosis ($P < .025$). Otherwise, there were no significant differences.

volumes being lowest in women undergoing BTL or tubal reanastomosis and highest in women with adhesions, endometriosis, fibroids, and PID. The absolute (total) amount (concentration times volume) of PF M-CSF was significantly lower in normal women (those undergoing a BTL and/or reanastomosis) (Fig 2). The absolute (total) amount of PF M-CSF correlated very closely with the absolute (total) number of peritoneal macrophages (Fig 3).

Because the peritoneal cavity in women communicates through the oviducts to the uterus, vagina, and the environment, patent oviducts represent an avenue through which exogenous materials (eg, microorganisms or their constituents [such as endotoxin]) could gain contact with normal peritoneal macrophages and modulate their function (including the stimulation of M-CSF elaboration). However, there were no differences in the concentrations of PF or plasma M-CSF nor the total amount of PF M-CSF with regard to the tubal patency status. In plasma, the concentrations were $0.90 \pm 0.16$ and $1.00 \pm 0.07$ ng/mL (mean ± SEM) for women with closed and open tubes, respectively; in PF, the concentrations were $2.60 \pm 0.31$ and $2.50 \pm 0.14$ for women with closed and open tubes, respectively; and in PF, the total amounts of M-CSF were...
DISCUSSION

Infertile women are known to have increased numbers of peritoneal leukocytes, 90% to 95% of which are macrophages. The highest numbers are found in women with endometriosis.1,3 We and others have hypothesized that macrophages (or their products) may adversely affect fertilization by injuring gametes or the embryo.1,3,11 Conditions characterized by high numbers of peritoneal macrophages (eg, endometriosis) are often accompanied by "idiopathic" infertility.1,3,13,20 Importantly, peritoneal macrophage number correlates with oviductal macrophage number,15 documenting that the peritoneal macrophages have access to oviducts, the site of fertilization. Information from studies of women with open or closed oviducts suggest that oviductal macrophages derive from peritoneal macrophages that emigrate through the open fimbriae into the oviducts.15

While it has been assumed that the increased numbers of peritoneal macrophages in infertile women result from an influx of blood monocytes into the peritoneal cavity in response to some inflammatory stimulus, it is also possible that some of the increase is caused by local proliferation of macrophages in response to high levels of growth factors. In this study, we demonstrate that PF levels of M-CSF are significantly higher than plasma levels, and that the absolute (total) amount of PF M-CSF correlates closely with the absolute (total) number of peritoneal macrophages present. The source of the PF M-CSF is not known. Macrophages in the peritoneal cavity could elaborate the factor and cause the noted elevations. Alternately, the M-CSF could in part (or whole) come from peritoneal fibroblasts or mesothelial cells stimulated to elaborate M-CSF by products of macrophages.21,22 Also, uterine epithelial (endometrial) cells can elaborate M-CSF.23 Because PF levels of M-CSF were the same in women with open and closed fallopian tubes, it is unlikely that reflux of uterine cavity M-CSF (derived from endometrial cells) contributed substantially to the PF levels.

As a blood monocyte enters extravascular tissues, certain micro-environmental factors cause the cell to differentiate into a phenotypically different cell, the tissue macrophage. The factors causing this in vivo differentiation are not known. Peritoneal macrophages are phenotypically different than blood monocytes in several respects: they are larger and have more vacuolated cytoplasms,1,3 have increased ability to phagocytize normal sperm,1,3 have increased ability to mediate spontaneous tumor cell killing in vitro,27 have altered expression of Ig Fc receptors,28 are unable to cap and internalize major histocompatibility complex class II antigens,29 have enhanced tissue factor (thromboplastin) expression,29 and have diminished ability to express/ elaborate IL-1 activity and present antigen29 (Weinberg JB, unpublished). Clearly, peritoneal macrophages are "differentiated" mononuclear phagocytes as compared with blood monocytes. PF M-CSF could play a role in the differentiation process; this factor causes "differentiation" or "activation" of monocytes and macrophages as judged by several different criteria.29 Because M-CSF can serve as a chemotactic factor for monocytes,30 PF M-CSF could attract blood monocytes into the peritoneal cavity, and then M-CSF (alone or in combination with other PF factors) could cause differentiation of the blood monocytes into the phenotypically different peritoneal macrophages.

The controls of partitioning of M-CSF between plasma and tissue fluids are not known. High serum levels of M-CSF have been reported in patients with myeloproliferative disease31 and in plasma of patients with ovarian cancer.32 The greater than 2.5-fold higher PF M-CSF levels (compared with plasma levels) that we observed here suggest a high rate of production of M-CSF in the peritoneal cavity, and/or a selective retention of, and/or a concentrating mechanism for M-CSF in PF. Based on our findings of compartmentally regulated levels of M-CSF, we can assume that plasma or serum levels will not always accurately reflect levels in tissue fluids. This finding is important in evaluating M-CSF levels in various pathologic states.

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