Decrease in Peripheral Blood Polymorphonuclear Leukocyte Chemotactic Index in Endometriosis: Role of Prostaglandin E2 Release

GIUSEPPE G. GARZETTI, MD, ANDREA CIAVATTINI, MD, MAURO PROVINCIALI, MD, MONICA AMATI, MD, MARIO MUZZIOLI, BD, AND MARIO GOVERNA, MD

Objective: To investigate the effect of disease on peripheral blood polymorphonuclear leukocyte chemotactic index and natural killer cell cytotoxicity and to provide additional information concerning the cell-mediated immune function in endometriosis.

Methods: Chemotactic index of peripheral blood polymorphonuclear leukocytes, natural killer cell activity, and plasma estradiol (E2) and plasma prostaglandin (PG) E2 levels were evaluated in 46 women who underwent laparoscopy or laparotomy for pelvic pain, infertility, and/or benign adnexal masses.

Results: The 20 women (43%) with endometriosis showed a decrease in peripheral blood polymorphonuclear leukocyte chemotactic index, related to advanced disease stage (P < .001). A significant inverse correlation was observed between plasma PGE2 levels and chemotactic index in stage III and IV endometriosis (r = -.73, P = .004). Similarly, natural cytotoxicity was decreased significantly with respect to the stage of endometriosis (P = .004) and related inversely to plasma PGE2 levels (r = -.74, P = .003). A direct relationship was observed between PGE2 and plasma E2 levels (r = .59, P = .006).

Conclusion: Advanced endometriosis is associated with decreased peripheral blood polymorphonuclear leukocyte chemotactic index and natural killer cytotoxicity, which may be related to plasma PGE2 and E2 levels. (Obstet Gynecol 1998;91:25–9. © 1998 by The American College of Obstetricians and Gynecologists.)

In spite of more than 6 decades of intensive investigation, the pathogenesis of endometriosis is poorly understood. In recent years, alterations in both cell-mediated and humoral immunity have been observed by several investigators in monkeys and in women with endometriosis. Host immunologic dysfunctions have been invoked as an important factor in the development of this disorder. Previous studies have shown that affected women have increased concentration and activation of endometrial and peritoneal macrophages and polymorphonuclear leukocytes, which can mediate the release of growth factors, prostaglandins (PGs), complement components, and lymphokines with subsequent effect on monocyte-macrophage activity and natural killer cell cytotoxicity.

However, endometriosis is a systemic estrogen-dependent disease, and it seems important to investigate not only local modulation but also systemic immune status and possible mechanisms of reciprocal interaction; estrogens are important regulators of immune functions. In our previous study, we observed a significant relationship between decrease in peripheral blood natural killer cell activity and serum levels of estradiol (E2) and we suggested an immunoenocrine interaction in the progression of endometriosis. However, the immunologic effects of estrogen may involve not only natural cytotoxicity but other mechanisms of immune response. Recently, Ito et al showed that estrogens modify the activity of macrophages and polymorphonuclear leukocytes during the normal menstrual cycle and negatively influence the inflammation process. This effect of estrogens might be due to a modulation of PG release; E2 increases the in vitro production of PGs by human endometrium fibroblasts. Prostaglandins are known to inhibit many functions of the immune system, including the chemotactic activity of peripheral blood polymorphonuclear leukocytes, and the cytotoxicity of natural killer cell activity.

In the present study, we analyzed chemotactic index of peripheral blood polymorphonuclear leukocytes and natural killer cell activity in patients with endometriosis, with respect to plasma PGE2 and E2 levels. The goal...
was to provide additional information concerning the cell-mediated immune function in endometriosis.

Materials and Methods

A cohort of 20 women with endometriosis, between 20 and 37 years of age (median 32 years) and parity between 0 and 2 (median 1), were recruited consecutively from a selected group of 46 patients who underwent laparotomy or laparoscopy for pelvic pain, infertility, and/or benign adnexal masses from the Department of Obstetrics and Gynecology, Ancona University, Ancona, Italy, between April 1995 and December 1995. The other patients had serious adnexal cysts in five cases (11%), dermoid cysts in two (4%), pelvic inflammation in four (9%), and no evidence of disease in 15 cases (33%). From our group, we excluded 1) women treated previously with GnRH agonist and/or any other medical treatment in the last 3 months (seven cases), 2) premenopausal women (two cases), and 3) women with chronic immune disease (two cases).

Samples of peripheral blood were drawn for endocrine and immune determinations during the early follicular phase of the cycle preceding laparoscopy or laparotomy. Blood was drawn in the standardized method, ie, on the same day of the cycle (day 5 from the onset of the menstrual cycle) and at the same time of the day. The early follicular phase was chosen to standardize the time of the menstrual cycle in which the hormonal changes and immune characteristics were evaluated.

At surgery, the patients with endometriosis were staged according to the revised 1985 American Fertility Society classification:41 seven patients (35%) had the revised American Fertility Society classification stage I or II endometriosis and 13 (65%) had stage III or IV. The controls were ten subjects with no evidence of endometriosis and/or other disease, recruited from a selected group, matched for age and parity, and with no history of infertility.

Peripheral blood mononuclear cells were fractionated on Hypaque-Ficoll (Pharmacia, Uppsala, Sweden) and separated by density gradient centrifugation (400 × g, 30 minutes). Cells from the interface of the gradients were washed twice with phosphate-buffered saline (Ca++ and Mg++ free; Gibco, Life Technology Inc., Gaithersburg, MD) resuspended in RPMI 1640 (Gibco) containing penicillin (100 U/mL), streptomycin (100 ug/mL), and 10% fetal calf serum (Gibco) (complete medium) at a concentration of 1 × 10⁶/mL. Viability was always greater than 98% as determined by trypan blue exclusion.

Natural killer cell activity of peripheral blood lymphocytes was determined by target cell retention of the fluorescent dye carboxyfluorescein diacetate as previously reported.15 The natural killer sensitive cell line K562 was used as the target cell. Lytic units (20/10⁶ cells) were calculated using linear regression analysis; one lytic unit corresponds to the number of effector cells required to produce 20% of specific lysis.

From another aliquot of blood, polymorphonuclear leukocytes were isolated by gradient centrifugation with Mono Poli Resolving Medium (Flow Laboratories, Irvine, Scotland) according to the procedure of Ferrante and Thong.16 Cells were washed twice with Hanks’ balanced salt solution (HBSS, Flow Laboratories) and suspended at a concentration of 2 × 10⁶ cells/mL in RPMI 1640 (Flow Laboratories) as a working suspension. Chemotactic activity was measured using a blindwell Boyden chamber (BM, Agrate Brianza, Italy), in which cells migrate through a micropore membrane. As previously described,17-19 0.5 mL of the working suspension was centrifuged against a 5-μm-pore-size cellulose acetate and nitrate filter (Millipore Corporation, Bedford, MA) using a cytocentrifuge (Shandon Scientific Co. Inc., Sewickley, PA). Then the filter was placed into the chamber with the cells laid on the upper surface.

The upper compartment of the Boyden chamber was filled with RPMI 1640, the lower one was filled with the Formil peptide (Sigma-Aldrich, Milano, Italy), dissolved at a concentration of 10⁻⁸ M in a solution of RPMI 1640 containing 2% human albumin. After incubation at 37°C in 5% CO₂ in air for 3 hours, the filters were removed, fixed, and stained. The total number of cells that had migrated completely through the filter within ten random microscopic fields (400×) were counted. The chemotactic index was calculated according to Hill et al20 with this formula: Polymorphonuclear leukocytes in 10 random fields/polymorphonuclear leukocytes delivered to the filter in millions. Each experiment was performed in triplicate and the mean value of the chemotactic index was calculated.

Preliminary experiments were carried out to check the cytocentrifugation of suspended cells onto the upper surface of the filter, which might reduce their number. A texture analysis system (TAS; LEITZ, Milano, Italy) was used to count the cells deposited on 20 filters through cytocentrifugation; the average number of polymorphonuclear leukocytes deposited on each filter was 802.52 ± 61.23.

Plasma levels of PGE₂ were detected by enzyme-linked immunosorbent assay (BIOTRA; Amersham Life Science, London, England) according to the manufacturer's directions. For the E2 assay, peripheral blood was collected in tubes containing ethylenediaminetetraacetic acid (1.5 mg/mL) and centrifuged within 4 hours of sampling. Each plasma specimen was stored at −20°C until assay. The E2 (Direct I125 Estradiol; Pantex, Santa Monica, CA) concentrations were evaluated by com-
Table 1. Peripheral Blood Polymorphonuclear Leukocytes in Patients With Endometriosis and in Controls

<table>
<thead>
<tr>
<th>Polymorphonuclear leukocytes</th>
<th>No. of women</th>
<th>Absolute no. per mm³</th>
<th>Percentage</th>
<th>Chemotactic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with endometriosis</td>
<td>20</td>
<td>3996.3 ± 943.6³</td>
<td>60.7 ± 3.2</td>
<td>72.4 ± 7.0⁹</td>
</tr>
<tr>
<td>AF5r stage I-II</td>
<td>7</td>
<td>3778.3 ± 814.2⁶</td>
<td>56.6 ± 6.8⁶</td>
<td>78.7 ± 3.6⁶</td>
</tr>
<tr>
<td>AF5r stage III-IV</td>
<td>13</td>
<td>4173.9 ± 1037.8⁴</td>
<td>63.0 ± 6.1³</td>
<td>69.1 ± 6.1³</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>4211.0 ± 1784.7¹</td>
<td>60.9 ± 1.7⁴</td>
<td>77.6 ± 3.5⁹</td>
</tr>
</tbody>
</table>

AF5r = revised 1985 American Fertility Society classification.

Data presented as n or mean ± standard deviation. Differences were not significant (P > .05) between a and ¹ (Student t test); between b and ² (Fisher exact test); among ³, ⁴, and ⁵ (analysis of variance); and among c, ⁶, and ⁷ (analysis of variance). Differences were significant between ³ and ¹ (Student t test, P = .04); and among ⁴, ⁵, and ⁶ (analysis of variance test, P < .001).

Table 2. Cytotoxic Activity of Peripheral Blood Lymphocytes in Patients With Endometriosis, With Respect to Revised 1985 American Fertility Society Classification Stage

<table>
<thead>
<tr>
<th>Cytotoxic activity (lytic units 20/10⁶ cells)</th>
<th>No. of patients</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with endometriosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF5r stage I-II</td>
<td>7</td>
<td>8.0</td>
<td>8.5</td>
<td>3.8</td>
</tr>
<tr>
<td>AF5r stage III</td>
<td>9</td>
<td>4.1</td>
<td>4.4</td>
<td>3.5</td>
</tr>
<tr>
<td>AF5r stage IV</td>
<td>4</td>
<td>2.4</td>
<td>2.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>4.2</td>
<td>5.4</td>
<td>4.0</td>
</tr>
</tbody>
</table>

AF5r = revised 1985 American Fertility Society classification; SD = standard deviation.

commercially available radioimmunoassay kits. The immunologic, PGE₂, and endocrine assays were performed blindly with respect to patient diagnosis. The data were analyzed statistically with the Student t test, analysis of variance test, Bonferroni t test, Fisher exact test, and correlation coefficient. The study size was established in terms of statistical power .90–.80 (α .05 and β .10–.20).

Results

Peripheral blood polymorphonuclear leukocyte chemotactic index in patients with endometriosis was decreased in comparison with that in controls (mean ± standard deviation 72.4 ± 7.0 versus 77.6 ± 3.5; Student t test, P = .04). A relationship was observed with respect to stage of disease (analysis of variance test, P < .001); polymorphonuclear leukocyte chemotactic index in patients with stage III or IV endometriosis was reduced significantly in comparison with that in patients with stage I or II endometriosis (69.1 ± 6.1 versus 78.7 ± 3.6; Bonferroni t test, P < .001). No significant difference in chemotactic index was observed between patients with stage I or II disease and controls (Bonferroni t test, P > .05). The reduction in polymorphonuclear leukocyte chemotactic index was independent of the number and relative percentage of leukocytes. Neither the absolute number nor the relative percentage of peripheral blood polymorphonuclear leukocytes was significantly different between patients and controls (Table 1). On the contrary, a significant inverse correlation was observed between plasma PGE₂ levels and polymorphonuclear leukocyte chemotactic index in stage III and IV endometriosis (correlation coefficient r = −.73, P = .004) (Figure 1). Patients with stage III or IV disease had higher plasma levels of PGE₂ than women with stage I or II disease (13.5 ± 10.3 versus 4.2 ± 1.9 pg/mL; Student t test, P = .03).

Similarly, cytotoxic activity of peripheral blood natural killer cells was decreased with respect to the stage of endometriosis and was related inversely to plasma PGE₂ levels (correlation coefficient r = −.74, P = .003). Patients with stage III or IV disease had lower levels of natural killer cytotoxicity than those with stage I or II disease (3.8 ± 3.5 versus 8.5 ± 3.8 lytic units; Student t test, P = .007) (Table 2).

A significant direct relationship was observed between plasma PGE₂ and E₂ levels (correlation coefficient r = .59, P = .006) (Figure 2). Patients with stage III

Figure 1. Inverse relationship between polymorphonuclear leukocyte chemotactic index and plasma prostaglandin E₂ levels in patients with stage I-II and III-IV endometriosis. AF5r = revised 1985 American Fertility Society classification.

Figure 2. Direct relationship between plasma prostaglandin E₂ and estradiol levels in patients with endometriosis. AF5r = revised 1985 American Fertility Society classification.

Garzetti et al Endometriosis and Prostaglandin E₂ 27
or IV disease had higher levels of plasma E2 than women with stage I or II (61.7 ± 32.4 versus 24.3 ± 4.8 pg/mL; Student t test, P = .01). In a multiple regression analysis, we evaluated the relative importance of disease stage (r = −.86, P < .001), PGE2 (r = −.70, P = .04), and E2 (r = −.72, P = .03) on polymorphonuclear leukocyte chemotactic index.

Discussion

We found peripheral blood polymorphonuclear leukocyte chemotactic index reduced in advanced endometriosis and related inversely to plasma PGE2 levels. A direct relationship between PGE2 and E2 levels also was found.

During the past decade, several reports indicated changes in cell-mediated immunity in women with endometriosis; these changes appear to be functional, do not affect general immune responses, and involve T cells,12,23 natural killer cells and their cytotoxicity,5,20,22 monocytes-macrophages,12,23 and polymorphonuclear leukocytes and their activity.12,23,24

All these cells have multiple functions; they secrete cytokines, including interleukins, interferon, tumor necrosis factors, and colony-stimulating factors, as well as several other substances, including growth factors, enzymes, PGs, and complement components able to modulate immune response. In particular, the polymorphonuclear leukocytes and monocytes-macrophages play a central role in the maintenance of humoral and cell-mediated immunity and seem to be affected in endometriosis. Secretory products of monocytes-macrophages can positively or negatively modulate activities of other immune cells, and polymorphonuclear leukocytes and monocytes-macrophages may play a role in the recognition of aberrant or misplaced self cells.12

In the present study, we found peripheral blood polymorphonuclear leukocyte chemotactic index reduced in women with advanced-stage endometriosis, independent of a reduction in polymorphonuclear leukocytes. This finding suggests a negative modulation of immune reactivity in advanced disease. Additionally, this finding is in agreement with a previous observation23 that cytotoxicity of peripheral polymorphonuclear leukocytes and peritoneal macrophages to culture hepatoma cell lines were unchanged or increased in mild endometriosis but decreased in severe disease.

We have investigated further the mechanisms responsible for the diminished polymorphonuclear leukocyte chemotactic index in endometriosis. We have shown a suggestive relationship among chemotactic activity, plasma PGE2 levels, and advanced-stage disease. In patients with advanced endometriosis, we observed lower values of polymorphonuclear leukocyte chemotactic activity and increased levels of PGE2 compared with women with less-advanced disease. Similarly, the inverse relationship between decrease in natural killer cell activity and PGE2 levels supports the correlation between polymorphonuclear leukocyte activity and natural killer homeostasis in the regulation of endometriotic spread.

Prostaglandins of the E series are known to inhibit many functions of the immune system, including polymorphonuclear leukocyte and monocyte-macrophage chemotactic activity, natural cytotoxicity, and T-lymphocyte proliferation.11-13 In particular, PGE2 has been shown to suppress mononuclear cell immune function through different mechanisms as well as through inhibition of interleukin-2 production mediated via the adenosine 3',5'-cyclic monophosphate, downregulation of major histocompatibility complex class II gene products, and blockade of the expression of transferrin and interleukin-2 specific receptors on activated lymphocytes. Previous studies showed significant increased concentrations of PGE2 in endometriosis, particularly in peritoneal fluid.26-28 Given that PGE2 suppresses these immune reactivities and its production is increased in stage III and IV endometriosis with depressed polymorphonuclear leukocyte chemotactic index and natural cytotoxicity, it may constitute an important downregulator of monocyte-macrophage, polymorphonuclear leukocyte, and natural killer cell activities. Interventions targeted at eicosanoid metabolism may be effective in advanced endometriosis. In particular, interferon-γ and indomethacin have shown an inhibiting effect on PGE2 synthesis or secretion by activated monocytes, and in optimal concentrations they abolish the suppressive effect of PGE2 on polymorphonuclear leukocyte and natural killer cell activities.29,30

Endometriosis is an estrogen-dependent disease,6 and all the immune modifications probably occur in the presence of estrogenic stimulation. A progressive decrease in chemotactic activity of polymorphonuclear leukocytes and monocytes was found after incubation with increasing concentrations of E2.10,21 Additionally, in vitro studies with long-term monolayer cell cultures of human endometrium showed that E2 increases the production of PGE2 in a dose-dependent manner.11

In the present study, we found a significant relationship between E2 and PGE2 plasma levels. Peripheral blood polymorphonuclear leukocyte chemotactic activity and natural killer cell activity possibly underwent a suppression as a consequence of increased PG secretion in an estrogenic habitat. These findings are in agreement with our previous report of a relationship between natural immune reactivity and estrogen levels in...
advanced endometriosis. However, the precise role of cell-mediated immune reactivity in the pathogenesis of endometriosis is not yet defined. Thus, the involvement of polymorphonuclear leukocyte and natural killer cell functions may be the effect of advanced disease rather than a cause of endometriosis progression.

References


Address reprint requests to:
Giuseppe G. Garzetti, MD
Department of Obstetrics and Gynecology
University of Ancona
Azienda Ospedaliera "G. Salesi"
via F. Corridoni 11
60123 Ancona
Italy

Received May 5, 1997.
Received in revised form August 25, 1997.
Accepted September 11, 1997.

Copyright © 1998 by The American College of Obstetricians and Gynecologists. Published by Elsevier Science Inc.