Endometrial Immunofluorescence Associated with Endometriosis and Pelvic Inflammatory Disease

DAVID KREINER, M.D., FRANK B. FROMOWITZ, M.D., DAVID A. RICHARDSON, M.D., AND DANIEL KENIGSBERG, M.D.

Reprinted from August 1986 Fertility and Sterility

PUBLISHED MONTHLY BY THE AMERICAN FERTILITY SOCIETY, BIRMINGHAM, ALABAMA
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Endometrial immunofluorescence associated with endometriosis and pelvic inflammatory disease

David Kreiner, M.D.; Frank B. Fromowitz, M.D.; David A. Richardson, M.D.; Daniel Kenigsberg, M.D.
Departments of Obstetrics and Gynecology and Pathology, University Hospital, Stony Brook, New York

Anti-immunoglobulin G (anti-IgG) staining in the endometrium by immunofluorescence has been associated with endometriosis. To investigate this phenomenon further, we took endometrial samples from 42 patients who underwent laparoscopy or laparotomy, which were tested for immunofluorescence. Fluorescein-labeled anti-IgG was incubated with tissue samples. Of 18 patients with documented endometriosis, 16 had positive immunofluorescence (89% sensitivity). Of 24 patients with no evidence of endometriosis, 9 had false-positive immunofluorescence and 15 had negative immunofluorescence. Of the 9 false-positive samples, 8 had evidence of old pelvic inflammatory disease. In the absence of this condition, there was only one false-positive study for immunofluorescence. The implications of these findings in terms of the pathophysiology of endometriosis-associated infertility is that it may be an immune-mediated process. With regard to diagnosis, the high predictive value of endometrial immunofluorescent IgG may be a useful tool in indicating early laparoscopic examination of the infertile period. Fertil Steril 46:243, 1986

The pathophysiology of endometriosis and its role in decreasing fertility remain obscure. Many studies have explored peritoneal factors such as prostaglandins, macrophages, and antibodies, which may prove to play a role. Cases of severe disease can easily explain the cause of infertility by impeding oocyte pickup or transport from the ovary through the fallopian tube. However, mild or minimal endometriosis has been hypothesized to cause infertility through these peritoneal factors by any of the following mechanisms. Prostaglandins have been implicated in preventing mechanical ovulation, inducing luteolysis and altering tubal motility. Macrophages, which appear to be activated and in a greater number in endometriosis, have been shown to cause phagocytosis of sperm, impair sperm function, and increase androgen production, which may inhibit follicular maturation. Endometriosis also activates the complement system, as demonstrated by the presence of significantly more complement components C3c and C4 in the serum, peritoneal fluid, and endometrium. It appears to induce production of antibodies that are likewise found in the serum, peritoneal fluid, and endometrium.

To investigate further the presence of endometrial antibodies associated with endometrium...
which may have implications regarding an autoimmune mechanism in causing infertility, we performed endometrial biopsies on 42 patients who underwent laparoscopy or laparotomy. These biopsies were then examined for the presence of immunoglobulin G (IgG).

MATERIALS AND METHODS

Forty-two patients who were undergoing laparoscopy or laparotomy for sterilization, pelvic pain, infertility, or other gynecologic surgery underwent endometrial biopsy at the time of surgery. Eighteen patients had endometriosis, 10 patients had chronic pelvic inflammatory disease (PID), and 14 patients had no disease. All patients in whom a diagnosis of endometriosis was made had unequivocal findings by gross and/or histologic examination. Chronic PID was diagnosed by the appearance of peritoneal adhesions, hydrosalpinx, and/or plasma cells on endometrial biopsy.

Fresh tissue from endometrial curettages or biopsies were frozen for immunofluorescent study. Fragments of tissue that appeared tan and non-hemorrhagic were isolated, sectioned, and frozen in one of two ways. The first 24 specimens were frozen in Optimal Cutting Temperature—Tissue Tek II (OCT) medium (Lab-Tek Products, Naperville, IL) in a cryostat and then incubated with fluorescein-conjugated goat anti-human antisera (Kent Laboratories, Inc., Redmond, WA) to IgG at room temperature in a dilution of 1:32. The latter group of 19 specimens were snap-frozen in liquid nitrogen in OCT medium and incubated with the goat anti-human antisera to IgG in increasing dilutions of 1:32, 1:64, and 1:128.

In ten of the first group of endometrial specimens, known cases of endometriosis and PID were compared with tissue from patients known to be free of those disorders for determination of the extent of background staining. Positive staining was defined as uniform, bright stromal immunofluorescence in the form of discrete globules. Negative results were recorded if the staining was irregular in distribution or dull. Both methods provided reproducible results, though the higher dilutions were somewhat easier to interpret and were perhaps more applicable to a grading system.

Statistical analysis was performed by the chi-square method.

RESULTS

Endometrial biopsy sections from 18 patients with endometriosis, 10 patients with chronic PID, and 14 patients with no pelvic disease were examined with fluorescein-conjugated anti-IgG for the presence of IgG in the endometrium (Fig. 1). Sixteen of the 18 patients with endometriosis had positive IgG staining (88.8%) (Table 1). A statistically significant positive association of immunofluorescent IgG and endometriosis was found ($P < 0.0001$). Eight of 10 patients with chronic PID had IgG in their endometrium ($P < 0.001$), but only 1 of 14 (7.1%) patients with no pelvic disease had a positive test ($P < 0.001$). However, if patients with endometriosis or chronic PID are combined, they constitute 96% of the positive tests for endometrial IgG ($P < 0.00001$).

The results were then analyzed with regard to their sensitivity, specificity, and predictive value of the immunofluorescent IgG test as a diagnostic test for endometriosis alone or pelvic disease in general (Table 2). The test for endometriosis was 88.8% sensitive, but with a low specificity and

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>IgG +</th>
<th>IgG -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometriosis</td>
<td>18</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Chronic PID</td>
<td>10</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>No pelvic disease</td>
<td>14</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>25</td>
<td>17</td>
</tr>
</tbody>
</table>

*Immunofluorescent IgG is associated with endometriosis and chronic PID ($P < 0.00001$) (chi-square analysis).
Table 2. The Sensitivity, Specificity, and Predictive Value of the Immunofluorescent IgG Test

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Predictive value IgG+</th>
<th>Predictive value IgG-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometriosis</td>
<td>88.8%</td>
<td>62.5%</td>
<td>64.0%</td>
<td>84.2%</td>
</tr>
<tr>
<td>Endometriosis or chronic PID</td>
<td>85.7%</td>
<td>92.9%</td>
<td>96.0%</td>
<td>76.5%</td>
</tr>
</tbody>
</table>

predictive value reflecting the high incidence of endometrial IgG in patients with chronic PID. A negative result was 88.2% predictive for the absence of endometriosis.

As a potential diagnostic test for any pelvic or peritoneal disease, the test has a predictive value of 96%, 85.7% sensitivity, and 92.9% specificity for a positive test, though a negative test was only 76.5% predictive.

DISCUSSION

Recent studies6, 10-12, 14 on the possible association of endometrial autoimmunity and endometriosis are beginning to explore a hypothesis proposed by Weed12 that endometrial proteins found in the peritoneal cavity of endometriosis patients may be recognized by the host as "foreign" and trigger an autoimmune response. Evidence for this autoimmune response is increasing. There is a significant increase in antibodies, complement components, macrophages, and lymphocytes in the peritoneal fluid of patients with endometriosis.3-9 It is suggested that the endometrial proteins from the ectopic endometrium in these patients are activating the macrophages and triggering an immune response. The increased complement and anti-endometrial antibodies in the serum and complement and antibodies in the endometrium add further evidence supporting an immune mechanism directed against the endometrium. An endometrial effect of the complement and antibodies may be to create an environment unfavorable for nidation and continued growth of the embryo. The association of endometriosis, and mild disease in particular, with a higher spontaneous abortion rate is possible evidence for this.15 There is perhaps greater evidence, however, for the role of macrophages, stimulated by the endometrial implants, to cause infertility by prevention of fertilization8 and by sperm phagocytosis.7 Evidence of testosterone production by macrophages raises a possible additional role of macrophages in affecting follicular steroidogenesis.

A surprise finding in this study was the high incidence of positive IgG tests in patients with signs of old PID. These were patients who had pelvic adhesions, hydrosalpinx, and/or plasma cells on endometrial biopsy. These patients, presumably, had endometritis associated with their salpingitis, so that their endometria would be infiltrated with antibodies as part of the immune response to the infection. An alternative hypothesis is that the infected and damaged endometria may continue to leak endometrial antigens, resulting in the same immune response, including macrophage activation, one would see in endometriosis.

These data suggest therefore that the positive immunofluorescent IgG test may be a valuable predictor of laparoscopic findings and may therefore be useful in a workup for infertility or chronic pelvic pain. With the aid of a positive biopsy, one may be able to counsel patients regarding the very high likelihood of a positive finding on laparoscopy. This may effectively shorten the workup of such patients and avoid the use of unnecessary tests and therapies.

In summary, data are presented confirming the presence of endometrial antibodies in endometriosis. Its association with chronic PID as well allows the presence of endometrial IgG to be used as a diagnostic test for pelvic disease which might prove to have practical application in the clinical evaluation of patients with infertility or chronic pelvic pain.

REFERENCES


