The endometria of patients with endometriosis show higher expression of class I human leukocyte antigen than the endometria of healthy women

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Objective: To compare the expression of class I human leukocyte antigen (HLA I) in endometrial samples from patients with and without endometriosis.

Design: Cross-sectional study.

Setting: Acute-care teaching hospital in Barcelona, Spain.

Patient(s): The endometriosis group included 32 patients for whom the only diagnosis during an operation was endometriosis. The control group included 20 women who underwent a laparoscopy and in whom no evidence of endometriosis or any other genital disease was seen.

Intervention(s): Samples of endometrium were obtained by curettage and immediately frozen. A pan–HLA I mouse anti-human IgG2a monoclonal antibody was used for immunohistochemical study.

Main Outcome Measure(s): Frequency of positive glandular and stromal cells was evaluated in each section.

Result(s): A significantly higher expression of HLA I in the endometriosis group than in controls, both in the glandular cells (median 100% vs. 80%) and in the stromal cells (median 60% vs. 20%), was observed.

Conclusion(s): Patients with endometriosis had a significantly higher expression of HLA I molecules in endometrial cells than did the controls. This could be a possible explanation for their higher resistance to natural killer cytolyis. (Fertil Steril 2006;85:78–83. ©2006 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, class I HLA, immunohistochemistry, natural killer cells

At this time, the hypothesis formulated by Sampson in 1927(1) is the most widely accepted when attempting to explain the origins of endometriosis. He proposed that endometrial cells reaching the peritoneum during retrograde menstruation were responsible for endometriotic implants. Retrograde menstruation, however, exists in 90% of all women, with patent tubes (2) and endometrial cells of menstrual fluid able to implant themselves (3, 4), and the incidence of endometriosis in premenopausal women is only 10%–15% (5).

The explanation for this could be defective natural killer cell (NK) activity in women affected by endometriosis, as observed by Oosterlynck et al. and Vigano et al. and confirmed by other authors (6–10). The immune system, particularly through NK, could be responsible for recognizing and eliminating misplaced autologous cells and antigens in non-affected women (11).

One of the known mechanisms of protection against NK cytolyis is the expression of class I human lymphocyte antigens (HLA I) on cells; the reduced expression of these molecules makes cells susceptible to NK destruction, because inhibitory receptors on NK are not activated.

The aim of this study was to compare the expression of HLA I in the endometria of women with and without endometriosis. An enhanced expression of these molecules in stromal or glandular cells could explain a higher resistance to NK destruction in this disease. This comparison has not previously been published in the literature.

MATERIALS AND METHODS

The endometriosis group included 32 patients, operated on in our department, who complained of pelvic pain, sterility, menstrual disturbances, or adnexal mass, and for whom the only diagnosis during the operation was endometriosis. The control group included 20 women who underwent a laparoscopy due to surgical sterilization, pelvic pain, or sterility, and in whom no evidence of endometriosis or any other genital disease was seen during the laparoscopic procedure after a careful survey of usual sites for endometrial growth. No history of autoimmune disease, malignant disease, hormonal
therapy, or immune therapy was admitted into either of the groups.

Samples of endometrium were obtained by curettage. Samples of endometriotic tissue were obtained during the operation in the endometriosis group. Informed consent was obtained from all of the patients before including them in this study. The study protocol was approved by the institutional review boards of the participating hospitals.

The endometriotic tissue was embedded in paraffin and stained with hematoxylin and eosin to confirm the diagnosis of endometriosis. The endometria for both groups were cut into blocks of approximately 1 cm³, partly embedded in paraffin, and stained with hematoxylin and eosin for histologic study and dating. Part was immediately frozen in liquid nitrogen for immunohistochemical study.

The monoclonal antibody used for HLA I staining was W6/32, a pan–HLA I mouse antihuman IgG2a monoclonal antibody (American T cell culture collection) kindly supplied by the Clinical Immunology Service of Hospital Germans Trias i Pujol, Barcelona. The peroxidase-antiperoxidase method was used, by which representative cryostat sections of 6 μ were cut and mounted on slides. They were fixed in acetone, then washed three times for 5 minutes in phosphate buffer solution (PBS), and immersed in 0.3% hydrogen peroxide to block endogenous peroxidase activity. The sections were washed again three times for 5 minutes in PBS and then preincubated with diluted normal universal serum for 15 minutes. Incubation with primary antibodies was performed for 1 hour at 37°C. After washing three times for 10 minutes in PBS, the sections were incubated for 30 minutes with a biotin-labeled universal immunoglobulin IgG. After washing in PBS, the sections were incubated with a streptavidin-biotin-peroxidase complex for 30 minutes. After washing the sections three times for 10 minutes with PBS, they were stained with diaminobenzidine and hydrogen peroxide.

Negative controls for immunostaining were prepared by replacing the first antibody with nonimmune serum IgG. Frozen sections of lymph nodes were used as positive external controls, and endothelial cells were used as positive internal controls.

Evaluation of staining was made by examining ten nonoverlapping fields per biopsy at a magnification of 400x. The frequency in percentages of positive glandular and stromal cells was evaluated in each section. The control group and the endometriosis group were compared, first considering all of the samples together, and then separately comparing samples in the proliferative phase and samples in the secretory phase. We also compared samples in the proliferative phase with samples in the secretory phase in each group.

Statistical analysis was performed using an SPSS package (SPSS, Chicago, IL). The Student t test, the χ² test, and the Mann-Whitney U test were used for analysis of the data.

RESULTS

A total of 15 samples from the control group and 25 from the endometriosis group were valid. Five control samples and seven endometriosis patients’ samples were illegible (tissue artifacts and poorly preserved) probably owing to deficient freezing. In the control group, the mean (SD) age was 33.9 (3.3) (range 27–41) years; 10 women were parous, and 5 were nulliparous. These 15 patients underwent a laparoscopy, for which the indications were surgical sterilization (n = 9), sterility (n = 2), and pelvic pain (n = 4). Four patients were in the proliferative phase of the menstrual cycle and 11 in the secretory phase.

In the endometriosis group, the mean age was 32.9 (7.3) (range 20–50) years; 12 patients were parous, and 13 patients were nulliparous. In 11 patients, surgery was performed through the laparoscopic approach and in 14 through laparotomy; the indications for operation included pelvic pain (n = 10), sterility (n = 6), adnexal mass (n = 6), and menstrual disturbances (n = 4). In this group, 13 women were in the proliferative phase of the menstrual cycle and 12 in the secretory phase. Following the revised American Fertility Society Classification, 1 patient had stage I endometriosis, 1 stage II endometriosis, 9 stage III endometriosis, and 14 stage IV. Patients in the control and endometriosis groups were comparable in age (t test = 0.680; P = .500), parity (χ² test = 1.320; P = .251), and number of samples in the proliferative and the secretory phases (χ² test = 2.462; P = .117).

The HLA I expression for each group is summarised in Table 1. When the whole study samples were considered, a significantly higher expression of HLA I in the endometriosis group than in controls, both in the glandular cells (Mann-Whitney test = 115.0; P = .043) and in the stromal cells (Mann-Whitney test = 83.5; P = .003), was observed (Figs. 1 and 2).

When samples in the proliferative phase were considered, the median of HLA I expression was higher in proliferating glands and in stromal cells in the endometriosis group than in control samples, but the difference was not statistically significant (Mann-Whitney test = 17.0; P = .350 for the comparison of expression in proliferating glands; and Mann-Whitney test = 13.0; P = .163 for the comparison of expression in stromal cells).

In the samples in the secretory phase, HLA I expression in the proliferating glands was higher in the endometriosis group, but the difference was not statistically significant (Mann-Whitney test = 41.5; P = .134). However, HLA I expression in stromal cells was significantly higher in the endometriosis group than in controls (Mann-Whitney test = 9.5; P = .000).

In control samples, HLA I expression in the proliferating glands was higher in the proliferative phase than in the secretory phase, but the differences were not statistically significant (Mann-Whitney test = 19.5; P = .753), whereas HLA I expres-
sion in stromal cells was higher in the secretory phase than in the proliferative phase though significant differences were not reached (Mann-Whitney test \( \text{H}_1 = 5.54; P = .022 \)). In the endometriosis group, HLA I expression in endometrial glands was similar in the proliferative and secretory phases (Mann-Whitney test \( \text{H}_1 = 12.5; P = .226 \)), although HLA I expression in stromal cells was significantly higher in the secretory than in the proliferative phase (Mann-Whitney test \( \text{H}_1 = 30; P = .000 \)).

### TABLE 1

**Results of HLA I Expression (Expressed as Percentage of Positive Cells)**

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Control group</th>
<th>Endometriosis group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Median (range)</td>
</tr>
<tr>
<td>All samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glands</td>
<td>15</td>
<td>80 (10–100)</td>
</tr>
<tr>
<td>Stroma</td>
<td>15</td>
<td>20 (10–70)</td>
</tr>
<tr>
<td>Samples in the proliferative phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glands</td>
<td>4</td>
<td>90 (10–100)</td>
</tr>
<tr>
<td>Stroma</td>
<td>4</td>
<td>10 (10–30)</td>
</tr>
<tr>
<td>Samples in the secretory phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glands</td>
<td>11</td>
<td>70 (20–100)</td>
</tr>
<tr>
<td>Stroma</td>
<td>11</td>
<td>20 (10–70)</td>
</tr>
</tbody>
</table>


### FIGURE 1

HLA Class I HLA expression from endometrium of patient 11 of the control group. Expression reached 30% in glandular cells and 20% in stromal cells. Original magnification 400×.
DISCUSSION

Since deficient NK activity has been found in women affected by endometriosis, it has been suggested that a particular resistance of their endometria against NK lysis could explain the development of endometriotic implants during retrograde menstruation (6, 7, 12). One of the determinants of the complex interaction between NK cells and their targets is the expression of HLA I molecules on the targets. In contrast to T-cells, NK spontaneously kill cells that weakly express HLA I (13).

In fact, several authors have observed low resistance to NK lysis in endometrial cultured cells, when the expression of HLA I molecules is reduced and, in contrast, high resistance when the expression is stimulated with gamma-interferon (14, 15). Surprisingly, we have found no references in the international literature comparing the expression of HLA I molecules between the endometria of women with endometriosis and the endometria of healthy women. All of the studies regarding the expression of HLA I molecules have been conducted on endometrial cultured cells.

In our study, we observed that the patients with endometriosis had a significantly higher expression of HLA I molecules than did the controls. Interestingly, this suggests that in normal women the expression of HLA I is low enough that during retrograde menstruation the endometrium that reaches peritoneum is nonresistant to NK destruction. In contrast, it would appear that the endometrial cells of women with endometriosis are resistant to NK lysis owing to their increased HLA I expression.

We have found no other studies in the literature comparing the expression of classical HLA I molecules in the endometria of women with and without endometriosis. There was a study conducted by Hornung et al. (16) comparing the expression of HLA-G, a nonclassical HLA I molecule normally expressed in the trophoblast, in the endometria of a control group and the endometria of patients with endometriosis. In this study, no expression of HLA-G in any of the samples was observed, discarding the expression of this molecule as a possible explanation for endometriosis.

Some limitations of the present study should be acknowledged. The number of samples processed is relatively small, so that more studies are needed to add further evidence to our findings. On the other hand, the control group included patients with infertility and pelvic pain who may have had minimal endometriosis not documented at laparoscopy. This might result in overlap of results regarding HLA I expression. In addition, spontaneously shed menstrual endometrium (i.e., the endometrium that is supposed to implant) would be the ideal specimen. However, when the study was designed, difficulties in scheduling the surgical operations for particular dates was the reason to accept endometrial samples of any phase of the menstrual cycle, so the present results should be interpreted taking into account this fact. Despite these limitations, however, the statistically signifi-

FIGURE 2

HLA Class I HLA expression from endometrium of patient 18 of the endometriosis group. Expression reached 70% in glandular cells and 90% in stromal cells. Original magnification 400×.
The expression of HLA I molecules was first studied by Johnson and Bulmer in 1984 (17), but Komatsu et al. (18) were the ones to describe cycling changes in the expression of HLA I molecules during the menstrual cycle. In normal endometria, they found a higher expression of HLA I mRNA during the luteal phase than was found during the proliferative phase of the cycle, especially affecting the stromal component.

In our study, we found that the expression of HLA I molecules was higher in the endometriosis group than in the control group, and that in the endometriosis group the expression in stromal cells but not in glandular cells was higher in the secretory phase than in the proliferative phase. This suggests that the endometria of women with endometriosis shows higher HLA I expression but following the physiologic changes described by Komatsu et al. In the control group, however, significant differences between both phases were not observed, although HLA I expression in stromal cells was higher in samples of the secretory phase than in those of the proliferative phase. This finding, however, may be attributed to the low number of cases included in the control group.

When both groups were compared considering samples from each phase of the cycle separately, HLA I expression was consistently higher in the endometriosis group, although significant differences were only found for stromal cells in the secretory phase. Further studies with a large number of patients are necessary to confirm whether differences in HLA I expression between women with and without endometriosis occur only in the final phase of the menstrual cycle, especially affecting the stromal component of the endometrium. Another possibility is that differences occur during the entire menstrual cycle in both the glands and stroma, but the number of cases in this study was insufficient to detect such a difference.

Another important issue is to find out what induces this high expression of HLA I molecules in the endometria of women with endometriosis. HLA I expression in the endometrium is induced by alpha-interferon, beta-interferon, gamma-interferon, and tumor necrosis factor (14, 15, 19). Progesterone also induces HLA I mRNA expression in endometrial cultured cells (18). No differences in the immunologic factors mentioned have been found in the endometria of women with and without endometriosis (20). Even though some studies have found high progesterone levels in the follicular fluid from women with endometriosis, increasing with the severity of the disease (21), others have found low progesterone levels circulating in these patients (22). Further studies should be conducted to identify the influence of progesterone in the origins of endometriosis.

Finally, it is important to consider that this enhanced expression of HLA I has important therapeutic consequences. In a randomized study conducted by Acien et al. (23) using intraperitoneal alpha2b-interferon during laparoscopy, in order to enhance NK activity, followed by Gn-RH analogs, they found higher relapses in the group treated with interferon. The explanation for this could be that the effect of interferon simultaneously increased NK activity and HLA-I expression in the endometria, and possibly, also in the endometriotic implants, making them more resistant to NK lysis. Other immunotherapeutic approaches should be considered for the treatment of endometriosis.

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