Expression of integrins and E-cadherin in cells from menstrual effluent, endometrium, peritoneal fluid, peritoneum, and endometriosis

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Objective: To detect the expression of integrins and E-cadherin in cells from peritoneal fluid (PF), endometrium, menstrual effluent, peritoneum, and endometriotic lesions during the early follicular phase of the menstrual cycle.

Design: An immunohistochemical study.

Setting: Tertiary care university medical center.

Patients: Sixteen patients undergoing a diagnostic laparoscopy as part of a subfertility work-up. All patients had regular and ovulatory cycles.

Interventions: A laparoscopy was performed in the early follicular phase (days 2 to 5). Simultaneously, samples were taken from endometrium, menstrual effluent, and PF, and a representative biopsy of an endometriotic lesion was obtained. If endometriosis was not noted, a peritoneal biopsy was obtained instead.

Main Outcome Measures: The expression of cell adhesion molecules, including the integrin α2β1, α3β1, α4β1, α5β1, and α6β1 and E-cadherin, as determined by immunohistochemistry on frozen sections.

Results: All integrins tested could be detected in the endometrium samples and in endometriotic lesions. In menstrual effluent samples, positive staining for the integrins α2β1 and α3β1 was found in epithelial cells in 13 of 16 cases. Integrin α6β1 was detected in 11 of 16 samples, and integrins α4β1 and α5β1 were detected in 8 of 16 samples. In PF, integrin α3β1 was found in epithelial cells in 12 of 16 samples, integrin α5β1 in 5 of 16, and integrins α4β1 and α6β1 in 2 of 16. The antibody for E-cadherin showed positive staining of epithelial cells in 6 of 16 menstrual effluent samples. All endometrial tissue samples showed positive staining for E-cadherin. In PF, E-cadherin was detected in the epithelial cells of one sample. One peritoneum biopsy revealed positive staining for E-cadherin.

Conclusion: Integrins α2β1, α3β1, α4β1, α5β1, and α6β1, and E-cadherin, important cell adhesion molecules, are expressed in endometriotic lesions and in cells and tissues that are potentially involved in the development of endometriosis. These cell adhesion molecules could be involved in the shedding of endometrial tissue during menstruation and the attachment of endometrial tissue fragments to the peritoneum.

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Key Words: Endometriosis, integrins, cadherins, cell adhesion molecules, menstrual effluent, peritoneal fluid, endometrium

Retrograde menstruation and peritoneal adhesion of shedded endometrial tissue is an essential element in the pathogenesis of endometriosis, according to Sampson's classical implantation theory (1–3). The presence of endometrial tissue fragments in peritoneal fluid (PF) during the early

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follicular phase of the cycle of women with patent tubes has been demonstrated (4). As has been shown before, early endometriosis is invading the extracellular matrix; however, the mechanism of adhesion of endometrial tissue to the peritoneum is not clear (5). We assume that cell adhesion molecules, such as integrins and cadherins may be involved in adhesion of endometrial fragments, which are present in the PF, to the peritoneum. Integrins are a family of cell membrane glycoproteins consisting of an α and a β subunit that mediate cell-cell and cell-matrix adhesion (6, 7). Currently, more than 20 integrin heterodimers are known, which are composed of 1 of at least 14 different α and 1 of 8 different β chains (8). The majority of the integrins presently known bind to various extracellular matrix components and mediate cell-matrix interactions during cell adhesion to basement membranes and other extracellular matrices and during cell migration (8, 9). Some integrins are also involved in cell-cell adhesion, i.e., the subunits α2 and α3 (10). The largest number of integrins are members of the β1 or very late activation antigen subfamily (6, 8).

The integrins of the β1 family, with α chains α1 to α6 serve as receptors for matrixproteins laminin (α1 to 3, α6), fibronectin (α2 to 5), and type IV collagen (α1 to 3) (8, 9, 11, 12). The distribution pattern of the integrins in tissues and how these patterns are affected by disease is relatively unknown, particularly with respect to the female genital tract. The cells of most tissues express α1β1, α2β1, α3β1, and α6β1. The integrin α5β1 is expressed to a lesser extent in most tissues.

Caderhins belong to a group of calcium-dependent transmembrane glycoproteins (13). Each cadherin type has a unique, but wide tissue distribution that may vary during different stages of development. Caderhins mediate cell-cell interaction. Adhering processes that involve caderhins are homophilic. Cells adhere preferentially to cells that express the same caderhin. Caderhins are important constituents of adherens junctions where they are responsible for cytoskeletal organization. E-caderhin (also known as Arc-1, uvomorulin, and cell-CAM 120/80) is expressed in all proliferating epithelial cells derived from the ectoderm and the entoderm. Neural and mesodermal tissues do not express E-caderhin, with the exception of some components of the urogenital system and some mesothelial layers (13). E-caderhin is expressed in the cell-to-cell-boundaries of the endometrium (14). Caderhins play an important role in invasive processes and metastasis of tumor cells (12, 15–17). At present, the potential role of cell adhesion molecules in the development of endometriosis is largely unknown. If the assumption is correct that integrins and caderhins account for adhesion of endometrial cells to peritoneum, which may lead to endometriosis, these cell adhesion molecules should be expressed on endometrial cells that are shed into the peritoneal cavity. Therefore, our aim was to study the expression of cell adhesion molecules on cells that are potentially involved in the development of endometriosis. To this end the expression of integrins and E-caderhin was detected using immunohistochemistry on cells present in PF, the endometrium, menstrual effluent, peritoneum, and endometriotic lesions in the early follicular phase of the menstrual cycle.

MATERIALS AND METHODS

Sixteen patients underwent a diagnostic laparoscopy as part of a subfertility work-up. All patients involved gave informed consent. All women had a regular and ovulatory cycle, as demonstrated by ultrasound and an adequate rise in serum P in the luteal phase of the cycle. The laparoscopy, using a double puncture technique, was performed in the early follicular phase (days 2 to 5). At the start of the laparoscopy, menstrual effluent was collected from the vagina using a syringe. A sample of endometrium was obtained by using a Probes endometrial sampling device (Gynetics, Oisterwijk, The Netherlands). Peritoneal fluid was collected immediately after introduction of the second trocar. Subsequently, a representative biopsy of an endometriotic lesion was obtained using a biopsy forceps, and in two patients an additional biopsy was obtained from unaffected peritoneum. When endometriosis was identified, it was staged according to the revised American Fertility Society (AFS) classification (18). When no endometriosis was diagnosed, a peritoneal biopsy was obtained from the pouch of Douglas. The samples of menstrual effluent and endometrium were frozen in isopentane immersed in dry ice. The tissue biopsies were embedded in Tissue Tek OCT compound (Miles Scientific, Elkhart, IN) and also frozen immediately. The PF samples were centrifuged for 5 minutes. After removal of the supernatant, the pellet was frozen in isopentane in dry ice. All samples were stored at −70°C until analyzed.

Cryostat 4-μm-thick sections were prepared and mounted on slides. The sections were air dried and fixed with methanol at −20°C for 1 minute,
followed by an acetone dip at -20°C. Slides were washed three times for 5 minutes in phosphate-buffered saline (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. A series of mouse monoclonal antibodies (mAbs) was used including L-CAM for E-cadherin (Euro-Diagnostica B.V., Apeldoorn, The Netherlands), I0G11 for integrin α2β1, J143 for α3β1, HP2/1 for integrin α4β1, and SAM-1 for α5β1, respectively. Also, the rat mAb G0H3 for α6β1 was applied. Incubation with primary antibodies against integrins and caderhin was done in appropriate dilutions for 60 minutes at room temperature. For staining with the mouse mAbs, a streptavidin-biotin complex method was used. After washing three times for 10 minutes in PBS, the sections were incubated with 30 minutes with biotin-labeled sheep anti-mouse immunoglobulin (IgG (Amersham Nederland B.V., Den Bosch, The Netherlands). After washing in PBS, the sections were incubated with a streptavidin-biotin-peroxidase complex (Dako A/S, Glostrup, Denmark) for 30 minutes. For staining with the rat mAb G0H3, the slides were washed three times for 10 minutes and then incubated for 60 minutes with rabbit anti-rat IgG conjugated with horseradish peroxidase (Dako A/S). Antibody binding was visualized using 3'-3-diaminobenzidine and hydrogen peroxide. The slides were counterstained with hematoxylin, and stained slides were dehydrated through alcohols, cleared in xylene, and mounted in Entellan for light microscopy. Positive staining was defined as immunoreactivity at the periphery of the cell. Negative controls included sections stained without the primary antibody, using PBS instead. Positive controls consisted of known positive samples of colon and endometrium.

RESULTS

Endometriosis was identified visually and confirmed histologically in eight patients and classified as stage I according to the revised AFS classification. All patients had patent tubes. In all PF samples a red color was noted, and red blood cells were identified. Table 1 summarizes the results of the immunohistochemical staining with the various mAbs against integrins in menstrual effluent, endometrium, PF, peritoneum, and endometriotic lesions. Figure 1A to G illustrates the staining patterns of the various mAbs used.

Ten peritoneal biopsies were tested: 8 from patients without endometriosis and 2 from patients who also had endometriotic lesions biopsied. Antibodies against integrins α2β1, α3β1, showed positive staining in the menstrual effluent samples from 13 patients. Integrin α5β1 was detected in 11 of 16 samples. The integrins α4β1 and α6β1 were found in only 5 of 16 samples. No relation was found between the presence or absence of these two in individual patients. In the endometrium samples, all integrins could be detected. Immunohistochemistry with anti-integrin α4β1 demonstrated heterogeneous staining. In the individual endometrial samples, positively as well as negatively stained glands were observed. Furthermore, α4β1 was only detected in 12 of 16 cases.

In PF, integrin α3β1 was found in epithelial cells in 12 of 16 samples, whereas integrins α4β1 and

| Table 1 | Immunohistochemistry of Integrins of Cells From Menstrual Effluent, Endometrium, PF, Peritoneum, and Endometriotic Lesions in 16 Women With and Without Endometriosis* |
| Tissue | Endometriosis | MAb/antigen |
|---|---|---|---|---|---|---|---|
| Menstrual effluent | + | 7/8 | 7/8 | 2/8 | 7/8 | 2/8 |
| Endometrium | + | 8/8 | 7/8 | 7/8 | 8/8 | 8/8 |
| PF | + | 8/8 | 7/8 | 5/8 | 8/8 | 8/8 |
| Peritoneum | + | 3/8 | 5/8 | 0/8 | 5/8 | 1/8 |
| Endometriotic lesion | + | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 |

* Number of cases with positive staining per number of cases tested.
αβ1 were only found in 2 of 16. In one case, this was in the same patient. Integrin α4β1 was not expressed in the epithelial cells of patients without endometriosis but was present in 2 of 8 patients with endometriosis. No differences between patients with and patients without endometriosis were found in the expression of the other integrins on the epithelial cells in PF. In endometriotic lesions, all tested integrins could be detected. Integrin α5β1 did not show reactivity in two cases that were stained positively for α2β1, α3β1, and α6β1 and in one case that did not reveal positive staining for any of the other integrins tested, except for α4β1. Integrin α4β1 was found in 3 of 8 samples.

Table 2 summarizes the results of the immunohistochemical staining patterns with the anti-E-cadherin mAb in menstrual effluent, endometrium, PF, peritoneum, and endometriotic lesions. Figure 1H illustrates the staining pattern. The antibody for E-cadherin showed positive staining of epithelial cells in six menstrual effluent samples: three from patients with and three from patients without endometriosis. All endometrial tissue samples showed positive staining for E-cadherin. In PF, E-cadherin was detected in the epithelial cells of only one sample. In this particular patient, E-cadherin was also found in the endometrium and in an endometriotic lesion but not in a sample of menstrual effluent. In another patient, E-cadherin staining was detected in the endometriotic lesion but was absent in menstrual effluent and PF. Only one peritoneum biopsy revealed positive staining for E-cadherin. The two biopsies from patients with endometriosis did not show positive staining for E-cadherin.

**DISCUSSION**

Retrograde menstruation is considered an important factor in the development of endometriosis. Pathogenetically, endometrial tissue, which is shed into the abdominal cavity, should adhere to the peritoneal lining. The present study demonstrates the presence of integrins and cadherins in cells and tissues that are potentially involved in the development of endometriosis and in endometriosis itself. In endometrium, all integrins that were tested could be detected. This is in accordance with a study of Tabibzadeh (12). Integrin α4β1 was found to be expressed in the endometrium during the early follicular phase of the menstrual cycle and in a gland-to-gland variation. Tabibzadeh (12) could

**Table 2 Immunohistochemistry of E-cadherin With Cells From Menstrual Effluent, Endometrium, PF, Peritoneum, and Endometriotic Lesions in 16 Women With and Without Endometriosis**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Endometriosis</th>
<th>L-CAM</th>
<th>E-cadherin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menstrual effluent</td>
<td>+</td>
<td>3/8</td>
<td></td>
</tr>
<tr>
<td>Endometrium</td>
<td>+</td>
<td>5/8</td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>+</td>
<td>1/8</td>
<td></td>
</tr>
<tr>
<td>Peritoneum</td>
<td>+</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td>Endometriotic lesion</td>
<td>+</td>
<td>1/8</td>
<td></td>
</tr>
</tbody>
</table>

* MAb or antigen; number of cases with positive staining per number of cases tested.
detect integrin α4β1 only in the glandular epithelium in the midproliferative and midsecretory phases. Lessey and co-workers (10) found a1, an integrin we did not test, only to be present during the secretory phase of the menstrual cycle on glandular epithelial cells, and a uniform expression of α2, α3, and α6 throughout the cycle was noted. In contrast to our results, integrin α4β1 was not found in either phase of the cycle in their study, and the α5 subunit only was found in the mesenchyme, which is rich in fibronectin.

Epithelial cells in menstrual effluent did express integrins. Integrin α4β1 and α6β1 were less frequently found, and in PF this effect was even more pronounced. Both cases that showed positive expression for α4β1 revealed endometriosis, whereas in none of the cases without endometriosis α4β1 was found. This suggests a potential role for integrin α4β1 in endometriosis, but the small number of cases does not allow definite conclusions.

All endometrial samples showed E-cadherin expression. The epithelial cells in menstrual effluent revealed E-cadherin expression to a lesser extent. No major differences were found between patients with and patients without endometriosis. The peritoneum samples only showed E-cadherin expression in one case. This suggests that E-cadherin does not play a prominent role in an adhesion process leading to the development of endometriosis.

The finding in our study that the cells in PF showed less reactivity with mAbs against E-cadherin and the integrins α4β1 and α6β1 than to the other cell adhesion molecules is confusing. One explanation could be that cells of the endometrium to be shed lose their expression of certain cell adhesion molecules. To our knowledge, no data exist on a possible cycle dependency of E-cadherin expression, as is found for integrin α4β1. E-cadherin expression may be temporarily decreased, only to be re-expressed once the endometriotic lesion has been organized. From cancer research it is known that when E-cadherin expression is lost, cells lose their interconnection and shed to invade (16). Integrins may also be involved in tumor invasion and metastasis. Malignant transformation is associated with a change in integrin expression (6). Well-differentiated tumors tend to express more E-cadherin than less differentiated tumors. A comparable mode of E-cadherin expression behavior in the processes involved in the menstrual shedding of endometrium is, however, not a satisfactory explanation for the finding of E-cadherin expression in only 1 of 16 cases in epithelial cells of PF, on the one hand, and in 6 of 16 samples of menstrual effluent on the other hand. Another explanation could be a technical artifact. When applying an antibody directed against the intermediate filament proteins such as cytokeratins, important constituents of the intracellular cytoskeleton, a strong staining intensity throughout the cytoplasm of the cell can be expected. The reactivity of E-cadherin is, however, limited to the cellular boundaries. The integrin α6β1 is expressed at the basolateral surface of the cell and, hence, gives a subtle signal when seen on a single cell (Fig. 1).

The demonstration of cell adhesion molecules in menstrual effluent, endometrium, PF, as well as in endometriotic lesions is no strict evidence that endometriosis originates from endometrium by retrograde shedding of viable tissue fragments. The expression pattern of cell adhesion molecules indicates that the loss of cell adhesion properties may play a role in the shedding of endometrial tissue during menstruation and in the attachment of endometrial tissue fragments to the peritoneum. Effective cellular adhesion requires that a given cell coordinates the action of the various adhesion molecules. It is, therefore, not to be expected that the processes involved in the adhesion of shed endometrial tissue in the pathogenesis of endometriosis can be explained by the presence or absence of one single cell adhesion molecule expression. Alternately, it is possible that other cell adhesion molecules that we did not study play a role in determining which endometrial tissues will adhere and proliferate.

In conclusion, the present study shows that all cells that are involved in the pathogenesis of endometriosis possess the property to express cell adhesion molecules. Although the pattern of integrins expressed by cultured cells is not always identical to that expressed by the same cells in their tissue of origin, it is mandatory to study the adhesion process involved in the pathogenesis of endometriosis both in vivo and in vitro.

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