Epithelial cells in peritoneal fluid—Of endometrial origin?

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OBJECTIVE: Our purpose was to examine the immunohistochemical properties of epithelial cells in peritoneal fluid and to compare the staining characteristics with cells of endometrium, menstrual effluent, peritoneum, and endometriotic lesions.

STUDY DESIGN: Samples of menstrual effluent, endometrium, and peritoneal fluid and biopsy specimens of endometriotic lesions and peritoneum from 16 patients were examined. Monoclonal antibodies against vimentin, cytokeratin 18 and 19, and the monoclonal antibody BW495/36, staining an epithelial marker present in endometrium and absent in peritoneal epithelium, were used.

RESULTS: All but one sample of menstrual effluent and peritoneal fluid cells stained positively with antibodies against vimentin and cytokeratin 18 and 19. BW495/36 stained 14 of 16 menstrual effluent samples and nine of 16 peritoneal fluid cell samples. Endometriotic specimens showed staining with all markers. No major differences in staining properties were observed in menstrual effluent, endometrium, and peritoneal fluid cells between patients with or without endometriosis.

CONCLUSION: These results support the contention of transport of menstrual detritus to the peritoneal cavity in women with patent fallopian tubes.


Key words: Endometriosis, epithelial markers, endometrium, peritoneal fluid, menstrual effluent

Retrograde menstruation and peritoneal adhesion of endometrial tissue are essential elements in the pathogenesis of endometriosis, according to Sampson's classic implantation theory.7, 8 Menstrual effluent is composed of extracellular fluid, blood elements, and endometrial cells. The presence of blood in peritoneal fluid during the menstrual phase of the cycle has been observed.4, 5 Several groups of investigators have reported the presence of endometrial tissue in the peritoneal fluid of women with patent tubes, irrespective of the presence of endometriosis.6-11 However, the number of women with endometrial tissue in the peritoneal fluid was smaller (0% to 59%) than the reported incidence of bloody peritoneal fluid in women with patent tubes (up to 90%). Because of the methods used for detection (i.e., Papanicolaou staining), only clumps of cells, and not single cells, could be detected and recognized as originating from endometrial tissue. Recently Kruitwagen et al.9 have demonstrated the presence of viable endometrial cells in peritoneal fluid. These authors succeeded in culturing these cells in vitro, and their data strongly support an endometrial origin of epithelial cells in peritoneal fluid.

The aim of the current study was to detect the presence of single endometrial cells or tissue fragments in peritoneal fluid in the early follicular phase of the menstrual cycle by use of immunohistochemistry with antibodies to epithelial markers. Also, we wanted to compare the immunohistochemical staining properties of the endometrial tissue fragments in peritoneal fluid with those of cells present in endometrium, menstrual effluent, peritoneum, and endometriotic lesions.

Material and methods

A diagnostic laparoscopy was performed in 16 women as part of the subfertility workup. All women had a regular ovulatory cycle, as demonstrated by ultrasonography and an adequate rise in serum progesterone in the luteal phase of the cycle. Laparoscopy was performed in the early follicular phase (day 2 to 5). The protocol of the study was approved by the hospital's institutional review board, and all women gave informed consent. At the start of the laparoscopy menstrual effluent was collected from the vagina with a syringe. A sample of endometrium was obtained with a Probit endometrial sampling device (Gynetics, Oisterwijk, The Netherlands). The laparoscopy was performed by a double puncture technique. Peritoneal fluid was collected immediately after the introduction of the second trocar. When endometriosis was identified, it was staged according to the revised American Fertility

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Table I. Immunohistochemical reactivity patterns of various monoclonal antibodies with epithelial cells of menstrual effluent, endometrium, peritoneal fluid, endometriotic lesions, and peritoneum in 16 patients during early follicular phase of menstrual cycle

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Endometriosis</th>
<th>Monoclonal antibody or antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RV 202 vimentin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RCK 106 cytokeratin 18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RCK 108 cytokeratin 19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BW495/36 epithelial marker</td>
</tr>
<tr>
<td>Menstrual effluent</td>
<td>+</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>8/8</td>
</tr>
<tr>
<td>Endometrium</td>
<td>+</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>8/8</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>+</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>8/8</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>+</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>8/8</td>
</tr>
<tr>
<td>Endometriotic lesion</td>
<td>+</td>
<td>8/8</td>
</tr>
</tbody>
</table>

Number of cases with positive epithelial cells per number of cases tested. Endometriosis was found in eight patients. +, Positive; −, negative.

Society classification.13 Subsequently, a representative biopsy specimen of an endometriotic lesion was obtained with a biopsy forceps, and in two patients an additional biopsy specimen was obtained from unaffected peritoneum from the pouch of Douglas. When no endometriosis was detected, a peritoneal biopsy specimen was obtained from the pouch of Douglas. The samples of menstrual effluent and endometrium were frozen in isopentane immersed in dry ice. The tissue biopsy specimens were embedded in Tissue Tek ornitine carbamyl transferase compound (Miles Scientific, Elkhart, Ind.) and also frozen immediately. The peritoneal fluid samples were centrifuged for 5 minutes. After removal of the supernatant, the remaining pellet was frozen in isopentane in dry ice. All samples were stored at −70°C until analysis.

Immunohistochemical staining procedures. Cryostat sections 4 μm thick were prepared and mounted on slides. The sections were air-dried and fixed with methanol at −90°C for 1 minute, followed by an acetone dip at −20°C. Slides were washed three times for 5 minutes in phosphate-buffered saline solution and immersed in 0.3% hydrogen peroxide to block endogenous peroxidase activity. The sections were washed again three times for 5 minutes in phosphate buffered saline solution. A series of mouse monoclonal antibodies were used, including RV 202 for vimentin, RCK 106 for cytokeratin 18, and RCK 108 for cytokeratin 19, respectively.13 Also the monoclonal antibody BW495/36 was applied, recognizing an epithelial marker present in endometrial epithelium but not in mesothelium.14 Incubation with primary antibodies against vimentin and the cytokeratins was done in appropriate dilutions for 60 minutes at room temperature. After being washed three times for 10 minutes in phosphate-buffered saline solution, the sections were incubated for 60 minutes with rabbit antimouse immunoglobulin G conjugated with horseradish peroxidase (Dako A/S, Glostrup, Denmark). For staining with BW495/36, a streptavidin-biotin complex method was used. In brief, after incubation with the primary antibody the slides were washed three times for 10 minutes and then incubated for 30 minutes with biotin-labeled sheep antimouse immunoglobulin G (Amersham Nederland, Den Bosch, The Netherlands). After being washed in phosphate buffered saline solution, the sections were incubated with a streptavidin-biotin-peroxidase complex (Dako A/S) for 30 minutes. Antibody binding was visualized with 3,3′-diaminobenzidine and hydrogen peroxide. The slides were counterstained with hematoxylin, and stained slides were dehydrated through an alcohol series, cleared in xylene, and mounted in Entellan (Merck, Darmstadt, Germany) for light microscopy. Negative controls included the procedure with the primary antibody left out, with phosphate-buffered saline solution used instead. Positive controls consisted of known positive samples of colon and endometrium.

Results

Endometriosis was identified visually in eight patients, confirmed histologically in all of them, and classified as stage I according to the revised American Fertility Society classification in eight of eight patients. All patients had patent tubes. In all peritoneal fluid samples a red color was noted, and red blood cells and epithelial cells were identified at light microscopy. Occasionally endometrial tissue was recognized as clumps of gland-like structures but in most cases as single cells. No difference in the presence of endometrial tissue was observed in patients with or without endometriosis. Also, no major differences in the reactivity patterns of the monoclonal antibodies in cells of menstrual effluent, endometrium, or peritoneal fluid were found between patients with or without endometriosis. Table I


summarizes the immunohistochemical staining obtained with the various monoclonal antibodies in menstrual effluent, endometrium, peritoneal fluid cells, endometriotic lesions, and peritoneum. Fig. 1 illustrates the staining patterns of the various monoclonal antibodies used. Ten peritoneal biopsy specimens were tested, eight from patients without endometriosis and two from patients who also had biopsy of endometriotic lesions at a different site in the pelvis.

Antibodies to vimentin, cytokeratin 18, and cytokeratin 19 showed positive staining of all the epithelial cells of the menstrual effluent samples but one. In the negative sample no nucleus-containing cells could be recognized. BW495/36 stained the epithelial cells in 14 of 16 menstrual effluent samples. All endometrial tissue samples except one were positive for vimentin and for the cytokeratins. BW495/36 did not show positive staining in one endometrium sample, although endometrial glands and stroma could be recognized at light microscopy and the other epithelial markers stained positively.

In peritoneal fluid the epithelial elements were detected by positive staining for cytokeratin 18 and cytokeratin 19 in all samples but one. This one did not contain sufficient epithelial cellular material to allow adequate staining. In one case positive staining for the cytokeratins, but not for vimentin, was found. In nine of 16 peritoneal fluid samples positive staining was found with BW495/36. All endometriosis samples except one stained for all epithelial markers. In this particular lesion there was positive reactivity for antibodies against vimentin, cytokeratin 18, and cytokeratin 19 but not for BW495/36. Also, the endometrial tissue sample of this patient failed to show positive reactivity with BW495/36.
All the peritoneum samples but one showed positive staining for vimentin, cytokeratin 18, and cytokeratin 19 in the mesothelial cell layer. In one sample no reactivity for cytokeratin 19 could be found. No reactivity with any of the peritoneum samples was found for the monoclonal antibody BW495/36.

Comment

Retrograde transport of endometrial tissue and subsequent appearance in peritoneal fluid is considered to play a pivotal role in the pathogenesis of endometriosis. Retrograde menstruation can be demonstrated by showing in peritoneal fluid the presence of blood, endometrial tissue fragments, by their morphologic characteristics, or epithelial components, which express immunohistochemical characteristics similar to endometrium. In the current study during the early follicular phase of the menstrual cycle each peritoneal fluid sample was colored red and contained red blood cells irrespective of the presence of endometriosis. Sampson reported the occurrence of blood dripping from one or both fallopian tubes when a laparotomy was performed during menstruation. Halme et al. found a red color of the peritoneal fluid in 90% of women with patent tubes, suggesting the presence of blood. Only visual documentation of the color of the peritoneal fluid samples was carried out. It has to be emphasized that these observations merely suggest that peritoneal fluid samples contain blood during menses in women with patent tubes. Retti et al. concluded that the demonstration of blood-stained fluid in the pouch of Douglas at laparoscopy was inadequate for the demonstration of retrograde menstruation, because in their study only a weak correlation was found between blood staining of peritoneal fluid and the presence of endometrioid cells. Only the presence of small clusters of cells visually resembling endometrial glands and stroma in the smear made from peritoneal fluid and stained according to Papanicolaou was recognized by these authors as evidence for their endometrial origin.

Demonstration of the presence of endometrial cells in peritoneal fluid is an objective way to assess retrograde menstruation. Various investigators have reported the presence of endometrium-like tissue in the peritoneal fluid of women with patent tubes, irrespective of the presence of endometriosis. The proportion of women with endometrial tissue detected in peritoneal fluid varied considerably between the studies performed, because of phase of the cycle studied, the method used for detecting the endometrial tissue, and prior flushing of the tubes. With Papanicolaou staining, which was most often used, only clusters of cells will be identified and not individual cells, because this staining cannot differentiate single endometrial cells from mesothelial cells. This methodologic problem probably accounts for the difference in incidence between bloody peritoneal fluid and the detection of endometrial tissue in peritoneal fluid from women with patent tubes. In the current immunohistochemical study we studied the peritoneal fluid cell population itself to determine the incidence of the presence of endometrial cells in peritoneal fluid of women with patent tubes. Our study demonstrates the presence of epithelial tissue fragments in peritoneal fluid in the early follicular phase of the cycle of all women with patent tubes. No flushing of the fallopian tubes was performed before collection of the samples. We did not find a difference in the presence of epithelial components in peritoneal fluid samples from patients with and without endometriosis. Our findings suggest that transport of menstrual detritus with cellular components and tissue fragments to the peritoneal cavity is a physiologic phenomenon.

Fifteen of 16 peritoneal fluid samples contained cells that stained positively with monoclonal antibodies against cytokeratins, underlining their epithelial origin. Only nine of these 16 stained positively with BW495/36. This difference may be related to a loss of the epitope for BW495/36 on these cells. It is tempting to suggest that endometrial tissue, after reaching the abdominal cavity, is modulated by the active peritoneal fluid components, leukocytes, macrophages, and proteolytic enzymes, resulting in single epithelial endometrial cells rather than endometrial tissue fragments. This is supported by the fact that we only occasionally found endometrial tissue as gland-like structures in the peritoneal fluid. Consequently, staining for epithelial markers that react with epitopes on the plasma membrane rather than intracellular filaments will be less intense. When these single cells attach to the peritoneal lining or to the underlying basement membrane and develop into endometriotic implants, the reactivity of BW495/36 occurs again. Kruitwagen et al. have found viable endometrial cells in 79% of women with patent tubes when culturing the cellular components of peritoneal fluid in vitro. A second explanation for this phenomenon might well be a technical artifact. The intermediate filament proteins such as cytokeratins and vimentin are abundant constituents of the intracellular cytoskeleton. When an antibody is applied directed against one of these components, a strong staining intensity throughout the cell can be expected. The reactivity of BW495/36 is much more limited to the cellular boundaries, and its epitope has not yet been identified. The use of sections rather than smears may influence the detectability of this antigen. The antibody has been shown, however, to stain endometrial epithelium and not mesothelium and is therefore a strong discriminating agent. Monoclonal antibodies against cytokeratins cannot be used to discriminate between epithelial cells
from endometrium and endometriosis and from peritoneal mesothelium. Alternatively, these cells may not be derived from the endometrium and hence not have been shed by retrograde menstruation into the peritoneal cavity. However, intact mesothelium is an unlikely source of these free floating epithelial cells, because mesothelial cells have only been found in the peritoneal fluid after damage to the peritoneal lining. Furthermore, we cannot exclude the potential origin of the epithelial cells from microscopic endometriotic implants, because these cells would probably show the same epithelial marker expression.

In conclusion, our study shows that peritoneal fluid contains single epithelial cells, rather than endometrial tissue fragments, in women with patent tubes. Possibly endometrial epithelial cells are modulated in the peritoneal cavity after they have left the uterine cavity before becoming an endometriotic lesion. Our study supplies new evidence for the contention of reflux menstrual detritus playing an important role in the development of endometriosis in women with patent tubes.

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REFERENCES