IS-64 Modulation of Apoptosis of Polymorphonuclear Leukocyte by Plasma and Peritoneal Fluid from Patients with Endometriosis

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Objectives: Increased production of pro-inflammatory chemokine cytokines for polymorphonuclear leukocytes (PMNs) has been found in endometriosis, suggesting that changes of the immune system play an important role in the pathophysiology of this disease. The effects of plasma and peritoneal fluid from patients who have endometriosis on apoptosis of PMNs were investigated.

Methods: PMNs and plasma of peripheral blood were prepared from women with endometriosis (n = 25) and healthy control (n = 20). Peritoneal fluid from 10 patients was collected at the beginning of laparoscopy. Apoptotic changes of the cells were evaluated by morphological changes using Giemsa staining. Three hundred cells were counted per sample and the percentage of cells with apoptotic changes was obtained. DNA electrophoretic analyses were used to confirm apoptosis.

Results: Compared to healthy control plasma, plasma from patients who had endometriosis reduced the percentage of apoptotic cells from 56.6% to 27.6% in cultures of PMNs maintained in vitro when assessed at 24 hours. PMNs from patients had a significant delay in apoptosis rate as compared with control PMNs in the presence of their autologous plasma (32.8% vs. 56.3%). Pro-inflammatory chemokine, interleukin-8 (IL-8) inhibited apoptosis of control PMNs but did not change apoptosis rate of PMNs from patients (2.5% vs. 35.7%). Peritoneal fluid obtained from patients inhibited apoptosis of PMNs from both control and patient groups (10.5% and 2.8%, respectively) and the effect of peritoneal fluid on apoptosis was abrogated by anti-IL-8 antibody (10.3% without antibody vs. 2.6% with antibody). However, the percentage of apoptosis observed in the presence of patient plasma was not changed by anti-IL-8 antibody. Lipopolysaccharide (1µg) inhibited the apoptosis of PMNs from healthy control but it did not affect that from endometriosis patients.

Conclusions: These findings show that luminal factor(s) affecting PMN survival present in the peripheral blood of patients who have endometriosis and that delayed apoptosis of PMNs may be related with inflammatory responses in endometriosis.

IS-65 Shed Endometrium in Menstrual Fluid Behaves Similar to Tumor Cell in Invasion Process

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Objectives: The nature of adhesion, implantation and invasion of the endometrial cells into peritoneum still remains to be elucidated for pathogenesis of endometriosis. The purposes of this study were, firstly to observe adhesion site of the shed endometrium (SE) in menstrual fluid (MF) to intact epithelium using ammon, and secondly to evaluate morphological changes of adhesion site by duration of in-vitro culture and access the process of adhesion and invasion of SE.

Methods and Materials: The MFs were collected from five fertile women who had regular menstruation on the second or third day of the menstrual period. The fresh ammon was obtained from tampon pads delivered without any complication. The SE in each MF was collected by filtering and were placed on amniotic epithelium (AE) and cultured for 1, 3, 5 or 7 days. The adhesion sites of SE were observed under a stereomicroscope and prepared for electron-microscopic observations.

Results: After 1 day of culture, adhesion sites of SE were observed but most of them were detached during preparation for histology. After 3 days of culture, the endometrial cells were shown to adhere to the AE. After 5 days of culture, the endometrial fragment composed of epithelial cells (EEC) and stromal cells (ESC) was demonstrated to adhere tightly to AE. At mid portion of the adhesion site, some ESC invaded into extracellular matrix (ECM) of AE. After 7 days of culture, the adhered EEC was shown to spread out from the adhesion site and detach the amniotic epithelial cells. According to spreading of EEC, the endometrial stroma located inside of endometrial fragment was in contact with ECM, and a large number of ESC were invading.

Conclusion: The shed endometrium in MF could adhere to and invade the epithelium, even though it was obtained from fertile women without endometriosis. This process could be described the sequence of three steps (attachment, degradation and invasion) and might be similar to tumor cell invasion.

IS-66 Parallel Increase in the Expression of Matrix Metalloproteinases (MMPs) and Tissue Inhibitor of Matrix Metalloproteinases (TIMPs) in Endometriosis

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Objective: To study the activity of MMPs and TIMPs in eutopic and ectopic endometriosis. Methods: Endometrial tissue was obtained as controls from myoma patients (follicular phase, N = 9; luteal phase, N = 14). Ectopic and eutopic endometrial tissues were chosen from endometriosis patients (follicular phase, N = 8; luteal phase, N = 17). Paraffin blocks were cut of 3 mm in thickness for immunohistochemical studies of MMP-1, 2, 3, 7, 9 and TIMP-1, 2, 3. The intensity of staining was scored as negative (0), weak (1), moderate (2) or strong (3). The distribution area of staining was also evaluated as none (0), <5% (1), 5% - 50% (2), >50% (3). The staining scores of MMPs and TIMPs were obtained from intensity x distribution. In addition, the score of MMP-TIMP for each slide was also calculated to verify the relative strength. Results: In follicular phase, we observed significant elevations of MMP-1 staining and a parallel increase of TIMP-1, 2, 3 in both glandular and stromal part of endometriosis. In luteal phase, more MMP-2 expressions were noted in both gland and stroma of endometriosis than those in controls. Conclusions: We demonstrated that the endometrial tissue in endometriosis possessed more MMPs' activities. Although the TIMP's expression increases coincidentally, it may serve to balance the MMP activities rather than play a role of destruction.